ASSOCIATION BETWEEN GRK5 POLYMORPHISM AND POORLY CONTROLLED ASTHMA

DISSERTATION

SUBMITTED FOR

M.D. PHARMACOLOGY

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY

DEPARTMENT OF PHARMACOLOGY

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

PEELAMEDU, COIMBATORE- 641 004

TAMILNADU, INDIA

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PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

COIMBATORE

CERTIFICATE

This is to certify that this dissertation entitled **"Association between GRK5 polymorphism and poorly controlled asthma"** by Dr.R.Keerthana Brattiya, is a work done by her during the period of study in the Department of Pharmacology from June 2012 to May 2015, under the guidance of Dr.S.Ramalingam, M.D., Professor, Department of Pharmacology and Principal, PSG IMS&R.

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After due consideration, the committee has decided to approve the above proposal.

The approval is valid for one year.

We request you to intimate the date of initiation of the study to IHEC, PSG IMS&R and also, after completion of the project, please submit completion report to IHEC.

We hereby confirm that neither you nor any of your study team members have participated in the voting/ decision making procedure of the committee. The members of the committee who have participated in the voting/ decision making procedure of the committee do not have any conflict of interest in the referenced study.

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

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15/2/17

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February 16, 2013

To
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Ref.: Proposal titled: *'Association between GRK5 polymorphism and poorly controlled asthma'*

Sub.: Ethics Committee Approval for the study

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 29th January, 2013 in its full board review meeting held at College Council Room, PSG IMS&R, between 2.00 pm and 5.00 pm, and discussed your application to conduct the study entitled:

"Association between GRK5 polymorphism and poorly controlled asthma"

The following documents were received for review:

1. Duly filled application form
2. Proposal
3. Informed Consent forms in English and Tamil
4. Case report form
5. CV
6. Budget

The members who attended the meeting at which your study proposal was discussed are as follows:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
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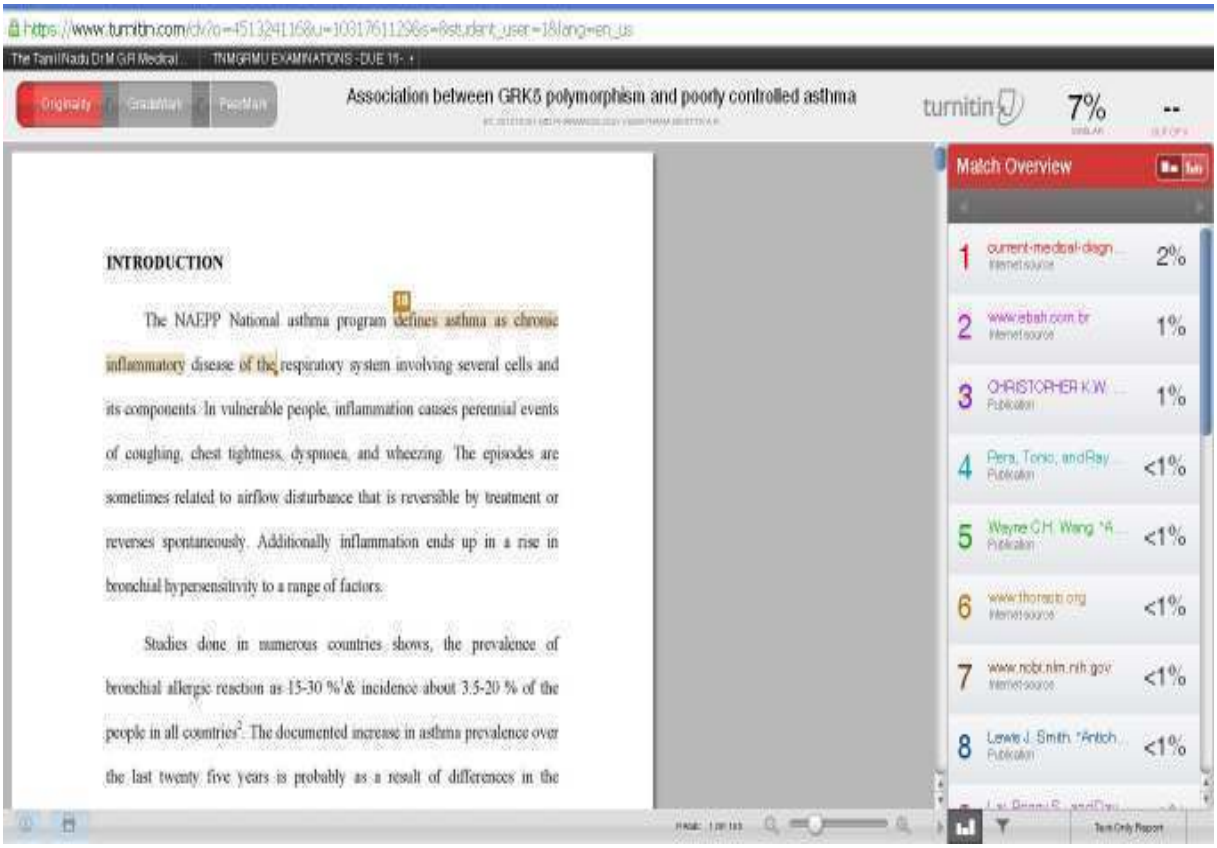
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INTRODUCTION
The NAEPP National asthma program defines asthma as chronic inflammatory disease of the respiratory system involving several cells and its components. In vulnerable people, inflammation causes perennial events of coughing, chest tightness, dyspnoea, and wheezing. The episodes are sometimes related to airflow disturbance that is reversible by treatment or reverses spontaneously. Additionally inflammation ends up in a rise in bronchial hypersensitivity to a range of factors.
Studies done in numerous countries shows, the prevalence of bronchial allergic reaction as 15-30 %[1]& incidence about 3.5-20 % of the people in all countries[2]. The documented increase in asthma prevalence over the last twenty five years is probably as a result of differences in the
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INTRODUCTION

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CONTENTS

ABSTRACT

TITLE

Association between GRK 5 polymorphism and poorly controlled asthma

BACKGROUND

Asthma is a chronic inflammation of the airways which are characterized with chest tightedness, wheezing, cough and dyspnoea. Despite the presence of effective asthma treatments and evidence-based management guidelines specializing in asthma management, several patients have bronchial asthma that's inadequately controlled. Several reasons are quoted for this poor management like patient non-compliance to treatment, poor inhalation technique, lack of response to medications, presence of co-morbid diseases, environmental exposure of hazards and genetic influence. Various genes are been researched for identifying the genetic factor. One of such unexplored genes is GRK 5 gene. This gene controls the kinase and turns off the receptor which results in decreased effect on administration of beta agonism.

AIM

To find out if GRK5 polymorphism has higher proportion of occurrence in poorly controlled bronchial asthmatic patients.

OBJECTIVE

1. To study the genetic association (GRK5-Leu41 polymorphism) and control (i.e. therapeutic response) in bronchial asthma.

2. To study the other risk factors associated with poorly controlled asthma.

METHODS

This was a cross sectional study with 160 patients. After getting informed consent from the patients they were divided into two groups (Well and Poor control) based on a self administered questionnaire – Asthma control test. Blood was obtained from these study participants and their genetic analysis was performed.

RESULTS

The prevalence of this polymorphism was found to be 14% in our population. But its association with poor control of asthma was not found to be statistically significant (p=0.822).Risk factors like known allergic history to dust, smoking history, climatic variation, history of GERD, regularity of treatment were all found to have statistical significance for poor control of asthma. In our study risk factors like progressing age, female sex, environmental hazard exposure, and exposure to pets were not statistically significant for poor controlled asthma.

CONCLUSION

The current study did not demonstrate a significant association between GRK5-Leu41 polymorphism and poor control of asthma.

Key words: Asthma, poor control, GRK 5 polymorphism

INTRODUCTION

The NAEPP National asthma program defines asthma as, chronic inflammatory disease of the respiratory system involving several cells and its components. In vulnerable people, inflammation causes perennial events of coughing, chest tightness, dyspnoea, and wheezing. The episodes are sometimes related to airflow disturbance that is reversible by treatment or reverses spontaneously. Additionally inflammation ends up in a rise in bronchial hypersensitivity to a range of factors.

Studies done in numerous countries shows, the prevalence of bronchial allergic reaction as 15-30 %[1]& incidence about 3.5-20 % of the people in all countries[2]. The documented increase in asthma prevalence over the last twenty five years is probably as a result of differences in the environment & life style as; modification in genetic makeup will take quite many generations to occur[3]. Worldwide, asthma cases are increasing at a rate of fifty per cent each decade, and in line with WHO (World Health Organization), by the year 2020, asthma, beside COPD (chronic obstructive pulmonary disease) can become the third leading reason behind death. A calculable three hundred million individuals within the world have asthma and there may be an extra one hundred million persons with asthma by 2025[4]

An epidemiologic multicentre study on the prevalence of asthma in Indian adults employing a uniform, valid and standardized methodology concluded a prevalence of 1.69-3.47 per cent[5] with increasing age, females, family history of respiratory illness, previous history of atopic allergy, lower socio-economic status and urban residence were considerably related to asthma[5]. In Delhi in urban center, parental smoking, paracetamol intake, exposure to cat, traffic pollution were found to be considerably related to current wheezing[6] whereas in youngsters aged 4-15 years in Chandigarh, a 7 % prevalence was observed[8]. India accounts for a 3rd of the world's asthma patients[7].

Globally, the cost related to respiratory illness exceeds those of TB and HIV/AIDS combined[9]. The economic value of asthma is extensive both in direct (hospital visits and price of pharmaceuticals) and indirect medical prices (sickness absenteeism and premature death). Developed countries expect to pay 1-2% of their health budget on Asthma[4].Thus the sickness burden is a lot of on the morbidity, mortality and conjointly on the economic status of the patient thus there's a necessity for higher management of the sickness status.

Despite the presence of effective asthma treatments and evidence-based management guidelines specializing in asthma management, several patients have bronchial asthma that's inadequately controlled.

Several reasons are quoted for this poor management like patient non-compliance to treatment, poor inhalation technique[10], presence of co-morbid diseases[11], triggers[12] like respiratory infections, specifically infectious agent[13], indoor allergens[14], environmental risky exposures[15], and lack of response to medications[16]. In addition, this data is supplemented in consideration with genetic modifiers of environmental exposures on expression of asthma. Various genetic alterations, SNP (single nucleotide polymorphism) are known within the factors affecting asthma and its effective management. E.g. ADRB2 gene, I L 17, TNF alpha ,ADAM 33, DPP10 , GRPA, etc[17].

One among such polymorphism that has not been effectively explored is GRK5 (G protein coupled receptor kinase 5).This study is to seek out if there's an association between GRK 5 polymorphism and poor control of asthma.

AIM

To find out if GRK5 polymorphism has higher proportion of occurrence in poorly controlled bronchial asthmatic patients.

OBJECTIVE

1. To study the genetic association (GRK5-Leu41 polymorphism) and control (i.e. therapeutic response) in bronchial asthma.

2. To study the other risk factors associated with poorly controlled asthma.

REVIEW OF LITERATURE

DEFINITION OF ASTHMA

The word "asthma" is derived from a Greek word meaning "panting" or "gasping for breath ".Asthma has been known to mankind since ancient times. Asthma in the ancient times was referred as a group of disorders that eventually manifested as periods of breathlessness often accompanied by a "wheeze".

The concepts of pathophysiology of this disease have changed from one school of thought to another. In 19th century Henry Hyde Salter[18] thought that both neural and vascular mechanisms were involved in development of asthma. He stated that "*The inflammation or congestion of mucous surface appears to be the stimulus that, through the nerves of the air tubes, excites the muscular wall to contract*". Sir William Osler thought psychogenic stimuli to be an important cause of exacerbation[19]. "All authors agree that there was, in a majority of cases of bronchial asthma, a strong neurotic element", This was written in his book "The principles and Practice of Medicine" in 1892.

In the latter part of 19th century, asthma was largely considered to be a psychoneurosis. However research done later tilted towards the

etiology toward the environmental agents. Pollen was demonstrated to be the cause of hay asthma by Charles Blackle[20]. Similarly the demonstration of smooth muscle antigenic sensitizations in animals[21] and the discovery of sensitizing proteins[22] in plants strengthened the role of environmental factors in chronic disease. Rackemann[23] classified asthma as extrinsic and intrinsic depending on the presence or absence of recognizable environmental triggers.

The CIBA guest symposium[24] was held in 1958 to arrive at common consensus over definitions, classifications and terminology of various respiratory disorders; "*Asthma refers to a condition in subjects with widespread narrowing of bronchial airways which changes in severity over short periods of time either spontaneously or under treatment and is not due to cardiovascular disease*"[24]. Approximately 25 years after CIBA symposium, the concept of generalized obstructive lung disease (GOLD) was introduced to accommodate both reversible and irreversible obstruction[25]. Asthma was defined in functional terms; however, the threshold of reversibility was not ascertained and this posed a diagnostic difficulty.

Around the same time there was another hypothesis originated from Netherlands, which considered reversible airflow obstruction of asthma and the largely irreversible airflow obstruction of smokers as the

two aspects of the same basic process. This hypothesis did not get acceptance in the United Kingdom and North America.

In 1990, in view of the high morbidity and mortality due to asthma in Britain, BTS also released guidelines for the asthma management[26]. In these guidelines, asthma is described as "Asthma is a common and chronic inflammatory condition of the airways whose cause is not completely understood. As a result of inflammation the airways are hyperresponsive and they narrow easily in response to a wide range of stimuli. This may result in coughing, wheezing, chest tightness and shortness of breath; these symptoms are often worse at night. Narrowing of the airway is usually reversible, but in some patients with chronic asthma the inflammation may lead to irreversible obstruction of airflow"[26].

However it took a century to reach a consensus regarding important components of the disease when in 1991, the National Heart Lung and Blood Institute formulated an expert panel group[27] which as a guide to describe asthma an identify treatment direction; put forth a working definition of asthma: "*Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role: In particular, mast cells, eosinophils, T lymphocytes, macrophages, neutrophils and epithelial cells. In susceptible individuals, this*

inflammation causes recurrent episodes of wheeze, breathlessness, chest tightness and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment .The inflammation also causes an associated increase in the existing bronchial hyperresponsiveness to a variety of stimuli. Reversibility of airflow limitation may be incomplete in some patients with asthma."[27, 28]

This definition was slightly modified in the 2007 Expert panel report 3[29] and was further modified in 2009 and the current definition as per the Global Initiative for Asthma (GINA) guidelines[30] is "*Asthma is a chronic inflammatory disorder of the airways in which many cellular events play a role .This chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness chest tightness and coughing ,particularly at night or early in the morning. These episodes are usually associated with airflow obstruction within the lung that is often reversible either spontaneously or with treatment*."

Over the past two centuries, defining asthma has been a herculean task owing to the lack of knowledge of its exact etiopathogenesis.

Although a universally acceptable definition of asthma has emerged, it is subject to further modifications based on future research.

PATHOPHYSIOLOGY

- The major asthma attack of respiratory disease embrace a variable degree of flow obstruction accompanied with edema of the airway, bronchoconstriction, increased cartilaginous tube secretion, airway inflammation and bronchial hyper responsiveness.

- Allergens that are inhaled results in an early-phase hypersensitivity with activation of IgE antibodies which is specific to allergen resulting in stimulation of mast cells & macrophages within the airway releasing unhealthy mediators like eicosanoids and histamines that provoke airway smooth muscular contraction, vasodilatation, secretion of mucous secretion, and plasma exudation within the airways. Reduced mucosal clearance is because of outflow of protein which ends up in an exceedingly stiffened, dropsical, engorged airway lumen and narrowing of the same.

- The late-phase inflammatory reaction happens six to nine hours when substance aggravates and indulges in accomplishment and stimulation of basophils, lymphocytes (T), eosinophils, macrophages and neutrophils.

- Unleashing of inflammatory mediators like leukotrienes, granule proteins, cytokines occurs when eosinophil migrates to the airways

- Activation of T-lymphocyte results in cytokines release from TH_2 cells that causes allergic inflammation (IL-4, IL-5, and IL-13). Also TH_1 cells manufacture IL-2 and IF-γ that are important for defense mechanisms in cell. Asthmatic inflammation could be due to imbalance of this TH_1 & TH_2 cells.

- Degranulation of mast cells in reaction to allergens leads to release of mediators like histamine; leucocyte, and neutrophil factors; Leukotriene C_4, D_4, and E_4; PGs; and Platelet Activating Factor. Sleek muscle contraction is caused by histamine and also plays role in mucosal wall thickening and secretion.

- Macrophages in alveoli unleash variety of acute phase reactants, as well as Platelet Activating Factor and LT's. Inflammation is further influenced by chemotactic factors from eosinophil & neutrophil.

- Bronchial hyper responsiveness & inflammation is caused by various mediators influenced by neutrophils like: PG's, TX, LT, and Platelet Activating Factor.

- The 5-LOX pathway is answerable for formation of cysteinyl LT's. LT's C_4, D_4, and E_4 are present throughout process of inflammation

within lung & turn out spasm, mucous secretion, microvascular permeableness, and airway edema.

- Bronchial animal tissue cells participate in inflammation by cathartic eicosanoids, peptidases, matrix proteins, cytokines, and nitric oxide. Epithelial shedding leads to heightened airway responsiveness, altered permeableness of the airway mucous membrane, depletion of epithelial-derived relaxant factors, and loss of enzymes liable for degrading inflammatory neuropeptides.

- The exudative inflammatory method and shedding of animal tissue cells into the airway lumen impair mucociliary transport. The gland size in bronchus increases & so goblet cell increase in number. Expectorated mucous secretion of patients with asthma tends to possess high viscosity.

- The airway is supplied by sympathetic, parasympathetic, and nor adrenergic system. The conventional tone of ASM is maintained by efferent vagal activity, and bronchial constriction is mediate by stimulation of vagus within tiny bronchi. Airway smooth muscle contains non-innervated β_2-adrenergic receptors that produce bronchodilatation. The nonadrenergic, noncholinergic system within the trachea and bronchi could amplify inflammation in respiratory disease by releasing nitric oxide[31].

Rise in numbers of mast cells in ASM are specific for bronchial asthma. Symptoms in bronchial asthma are attributable to ASM contraction, and so bronchodilators are needed as symptomatic relief drugs. The physiological hallmark of bronchial asthma is airway hyper responsiveness. Whether or not ASM is per se altered in bronchial asthma isn't confirmed. However augmented ability of ASM could influence the current bronchial hyper responsiveness. Inflammation additionally is also orchestrated by nerve fiber that controls TH_2 cells which regulate inflammation by eosinophils & immunoglobulin production by B lymphocytes. Bronchial epithelial tissue has a crucial part by discharge of number of inflammatory mediators &thru releasing growth factors to correct the injury caused by inflammation. This inflammation in bronchial asthma is mediated by discharge of many mediators[32]. Cytokine protein networks, together with chemokines and growth factors, play vital roles in orchestrating the inflammation method [33].

Asthma mainly characterized by a mode of inflammation mostly caused by immune gamma globulin (IgE)-dependent mechanisms. Genetic factors plays a crucial influence whether or not atopic allergy develops and a number of other genes are been identified[34]. Several genetic factors of bronchial asthma are also common to many allergic diseases[35]. In addition, environmental factors seem to be additional vital

in determinative whether or not an atopic patient results in bronchial asthma, though factors related to genetics could impart an effect on how severely illness is present and increase in inflammatory process.

Mast cells have an important role in causing bronchial asthma symptoms, while macrophages, TH_2cells & eosinophils are mainly concerned with inflammation that causes AHR. There is high fact that the cells of bronchi, together with epithelial cells of bronchus and ASM cells form a crucial means of mediators of inflammation. Several mediators of inflammation are important for bronchial asthma, together with lipid mediators & peptide mediators, cytokines, growth factors and chemokines. They play important role in specific accomplishment of inflammatory mediators in circulation, while cytokines mediate chronic type of inflammation. This chronicity may be the reason for morphological changes in airways, with subepithelial pathology, ASM hyperplasia, growth & mucous secretion. Pro-inflammatory transcription factors, like NF-kB and GATA-3 has main role in forming expression of genes related to inflammation.

Many internal mechanisms are there that balance the inflammation in asthma and a few proofs these could also be reduced in asthma. Owing to complexness in bronchial asthma medication which focuses on a cell / intermediator is less likely supply vital clinical advantage;

Beta2-Agonists don't seem to be solely the foremost acting bronchodilators; however beta agonists additionally inhibit plasma leak & mast cells, while corticosteroids inhibit many inflammatory process & also production of chemokines and cytokines.

RISK FACTORS

Asthma may be an advanced cluster of conditions, and efforts are created to outline phenotypes/endotypes based on age of onset, duration, severity, presence of hypersensitivity reaction and alternative factors. There are several modifiable and non modifiable risk factors for the patients and this will influence the episodes of exacerbation, hospital admission, frequency of medications etc. Factors embrace better-known allergens, environmental pollutants, smoking history, co morbidities and a lot of more.

Environmental triggers influence asthma at totally different times in life. And even the better-known risk factors could modify over time for a personal .This may ensue to sensitization following exposure or tolerance to the substance.

Short term studies of risk issue could counsel there's a rise in probability of asthma however constant risk factors is also related to a decreased risk once followed up for a longer time or the other way

around.

A wheezing trigger is something that irritates airways and causes symptoms. Triggers could embrace animal hair, exercise, food, medicines, spore or farming dusts, climatical changes and tobacco smoke.

OCCUPATION

Between two hundred and three hundred agents are encountered at work that is reportable to cause asthma attack through respiratory sensitization.[35, 36, 37]. Exposure to environmental tobacco smoke has been a predominant hazard and a few proof suggests that such exposure in adulthood will increase the danger of asthma attack[38].Workplace exposure to environmental tobacco smoke has been coupled with asthma attack .[39, 40, 41]

A study done in guinea pigs where the animals were gone through few respiratory pollutants like

- Endotoxin,
- Cotton Dirt,
- β-glucan and
- Tannin

Endotoxin recapitulated neutrophil adherence, tachypnea, and decreased airway conductance with exposure of cotton dirt, though toxin

didn't influence contraction of ASM which was isolated[42].A study in human volunteers who were healthy didn't show a dose-response changes in FEV_1 in relation with cotton dust[43].

A novel animal model was prepared for workers in cotton textile; toxins were used to assess difference in early with late responses. Significant changes in bronchial hyper-reactivity with 5 day exposure was seen, in comparison with redoubled bronchial airways resistance when 8 wks exposure. Which is often in line with the finding like cotton dirt could cause reversible then fastened air flow hindrance over time. Curiously, rising period in toxin exposure is related to risen pneumonic inflammation.

Also together with this the growth of dendritic cells & decrease in macrophages necessary for resolution of inflammation was found out, which can be a mechanism of chronic hyper reactive airway found in textile workers[44].

AIR POLLUTION

The mortality after first of 20th century was smogginess in United States of America & European Union, without ambiguity proved the link between pollution and airway illness. Pollutants which lead to increased risk of recurrence in wheezing patients included

- Sulphur dioxide (SO_2)
- Smoke
- Nitrogen dioxide (NO_2)
- Ozone (O_3)
- Hydrocarbon and
- Diesel

Dangers exposed by pollutants are assessed by seasonal outdoor concentrations & conjointly exposure of wheezing subjects individually and relation with different co-variances.

Proof of pollution implies that it should stimulate exacerbations in patients with asthma attack; however there is no proof to recommend like pollution starts asthma attack among people with antecedently good lungs[45]. Employing different type of epidemiologic methods, exposure and outdoor pollutants was connected to exacerbations symptoms by ER visits[46,47], hospital admissions[48,49], mortality[50,51], enhanced URT and LRT symptoms[52,53] and reduced lung function[54,55].

Studies done in controlled chamber conjointly allowed finding the airway response aggravated by inspiration of mobile dusts that provide amount of the propensity to cause exacerbation. Few studies done in populations of young and old asthmatics showed different response to

completely different pollutants, however ensuring general redoubled status in wheezing in comparison with non – asthmatic subjects[56].

FOOD

There are many case-reports demonstrating asthma attack are often triggered by foods, & is reduced by change in diet. Symptoms attributable to Immunoglobulin E-mediated hypersensitivity may occur if the food is not habitually eaten, leading to hypersensitivity reaction, which can be in the course of exacerbated asthma attack. Food habitually eaten may present as chronic asthma attack typically related to atopic eczema. In pediatric age group it present as asthma with classical sign some time with exacerbation of symptom[57].

Adults with above normal weight relation with disease understand that food plays important role in control of disease. A study of hundred thirty five adults asthmatics attending an hypersensitivity reaction clinic, seventy three had symptoms due to food, and sixty one managed to change their diet so improve their management of disease[58].

In a study Seventeen percent of adults. Who had food intolerance also was atopic[59]. Most reason behind food evoked asthma attack are discovered in infancy and are then typically associated with egg, flour, peanuts and cow's milk hypersensitivity. In spite of influence of foods in

acute attack, a study with placebo done with double blind in controlled setting is standard for identification response to foods. Such studies have shown that <1% of patients could have proof of acute attack due to food [60]. Hence general prevalence is around four to six percent for kids, less than one percent in adult patients.

GASTROESOPHAGEAL REFLUX

GERD is also an initiator of asthma attack; anti reflux medical care is also useful in selective patients with poorly controlled asthma as well as repeated exacerbation. Many researchers have related esophageal reflux with asthma attack which proves GERD is also a risk factor[61]. Mechanism is mostly due to exaggerated vagal stimulation due to acid stirred up receptors in esophagus, micro aspiration of acid within airway and enhanced ventilation when acid is exposed are the foremost possible causes. Regular anti reflux medical care is probably going to boost prognosis in twenty to seventy percent of adult patients[62, 63]. A study review done enclosed three ninety six cases reported that 9 of the entire twelve studies there was vital improvement in a minimum of one outcome or overall symptoms. However results weren't standard in studies[64].

NOISE

Noise pollution also influences changes in respiratory epithelium. Animals exposed to recordings of textile area noise almost daily/40 hrs/wk caused vital changes in bronchial ciliated cells at one month that stayed throughout study period; this could cause improper mucociliary clearance & cough[65].

SMOKING

Previous studies have disclosed that cigarette smoking will induce airway inflammation and increase airway responsiveness[66, 67, 68] and it's related to an asthma attack phenotype[69, 70]. Passive tobacco smoke exposure is related with onset of disease attack in early childhood[71], augmented emergency room visits for kids with asthma[72], and exaggerated respiratory symptoms among the overall population[73]. Though active and passive cigarette smoking is well-recognized risk factors for asthma, solely a little of people manifest asthma attack. Some have speculated that people could vary in genetic status to cigarette smoke[74, 75] however gene–smoking interactions haven't been examined in asthma or chronic obstructive lung disease. Increased airway mucosal permeability and nonspecific airway responsiveness have additionally been discovered in cigarette smokers with lung respiratory organ function[76, 77].

Exposure to tobacco smoke either through active smoking or by passive smoke cause and/or exacerbate an acute attack or sickness symptoms. For individuals exposed to passive smoke at their work the danger of developing adult onset asthma attack is alleged to be double that of these who aren't exposed to passive smoke. For individuals exposed to passive smoke at home the danger of developing asthma attack is 5 fold[78, 79]. The 2001 Health Survey for England found that being exposed to passive smoke for six or additional hours during a week have considerably multiplied risk of wheezing.

A study conducted in 2006 identified that regular cigarette smoking by adolescents will increase the danger of asthma in teenagers with no lifetime history of asthma or wheezing. Teenagers who preserved often were fourfold additional seemingly to develop asthma attack over succeeding eight years than nonsmokers[80].The airways of an individual with asthma attack are terribly sensitive to triggers. Tobacco smoke has been found to be a significant asthma attack trigger and it can even have an effect on the severity of an attack.

It is typically assumed and expected that individuals with asthma attack avoid smoking. However, a study in 2006 conducted in United Kingdom of Great Britain and Northern Ireland found that 24% of adults with asthma attack do have the habit of smoking. A Californian study in

2001 additionally found no distinction in prevalence of smokers with asthma attack (20.2%) and people without (18.8%).[81]

People with bronchial asthma who smoked and still smoke have worse symptoms and knowledge a lot of fast decline in pneumonic function than people who have asthma however don't smoke. Those individuals with asthma attack and who smoke are additional liable to chest infections as a result of the body isn't ready to get eliminate the smoke from lungs properly. Another study found that compared those with nonsmoking asthmatics, smokers had additional respiratory symptoms and had options almost like those found within the early stages of chronic obstructive pulmonary disease (COPD) [82].

People with asthma who smoke are doubtless to experience higher rates of hospitalization than those with asthma that don't smoke.[83] Together with diminished lung function, tobacco decreases the effectiveness of medication.

Quitting smoking has been shown to enhance the respiratory organ function of individuals with asthma attack. One study showed improved lung performance and a fall in mucus neutrophil (phlegm) levels once individuals quit smoking for six weeks compared to those that continuing to smoke[84]. Stopping smoking will improve the health of individuals with

bronchial asthma and thus each effort ought to be created to encourage those with asthma who smoke to prevent smoking.[82]

Research revealed at the end of 2003 concluded that passive smoke will cause asthma in adults[85] .This was supported in 2005 by the Royal college of Physicians. Analysis by the IARC shows the strongest causative impact of passive smoke exposure is chronic respiratory symptoms in adults. For individuals exposed to passive smoking at their locality, risk of developing adult onset bronchial asthma is double that of these not exposed. For folks exposed to passive smoking at home, risk of developing asthma attack is 5 folds.

OBESITY

Asthma & obesity are main issues distressing many people in world. It is commonly based on BMI. There are many studies done to spot the assorted risk factors and multiple mechanisms involving the relation for poor management of asthma attack and fatness. Majority of the studies imply that there's relation between obesity & poor management of BA; few studies recommend no association[86] .One review article in 2013 by Shannon Nonosad et al. has concluded that asthma attack and obesity express a significant relationship with difficult-to-control phenotype, which has impaired responses to controller drugs. Such set of patients are additional seemingly to possess a poor quality of

life, a lot of symptoms, exacerbations likewise more emergency medications. The augmented risk during this constitution is also as a result of alteration in inflammation from changes in levels of leptin, adiponectin, there will be high oxidative stress, and obesity elicited changes in volume of lung.

Another study by Carlos et al. conducted in United States population in 1999, concluded that BMI features a sturdy independent and positive association with the chance of adult onset asthma. Additionally the exacerbations and not only obese people it also discovered that, the chance of asthma is increased even in people with BMI 22.5 to 24.9[87].This level is below the quality clinical level of obesity definition and thus is unlikely to possess to have to any identification bias. And each woman enclosed during this study with weight considered being normal or an average was at a rather exaggerated risk. It's additionally a vicious cycle as a result of when patients become poorly controlled asthmatics their physical and day to day activity is been restricted and this successively results in increase in BMI of that patient.

GPCR & GRK 5

A wide range of extracellular signals, like

- Neurotransmitters

- Hormones
- Chemokines
- Light and
- Odorants

are sensed by G protein coupled receptors (GPCRs). Over 1000 types of this receptor are present. All GPCRs known so far has standard structure of seven membrane-spanning helices and which mediates signals from extracellular to intracellular by stimulating G proteins[88].

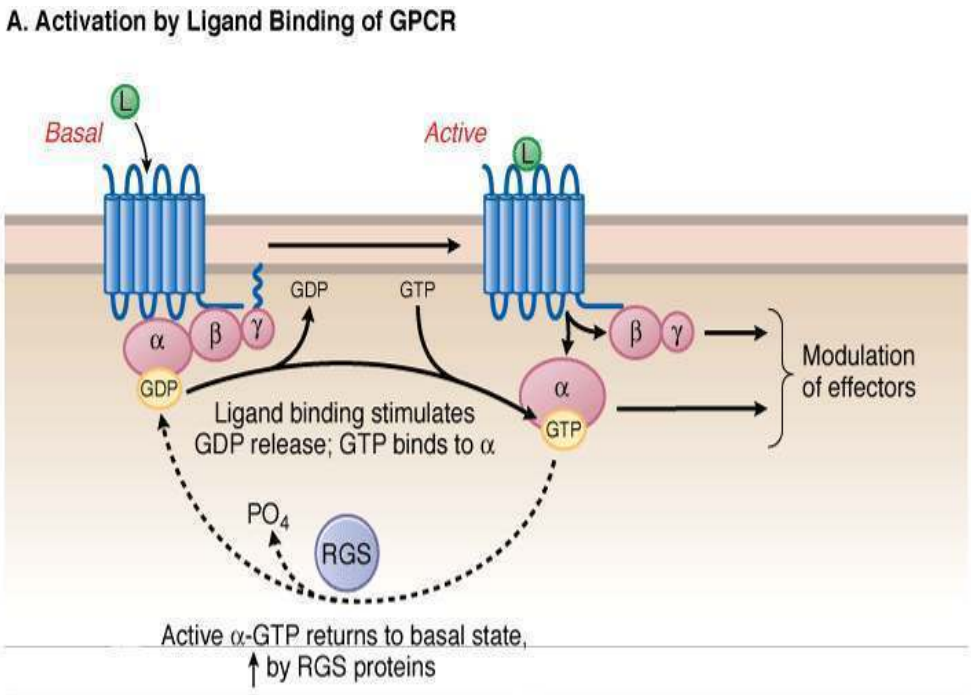
GPCRs span around plasma membrane like bundle of 7 α helices[89]. Humans express over 800 GPCRs which forms 3rd biggest group of genes in humans, with roughly half of these GPCRs dedicated to sensory perception (smell, taste, and vision). The remaining receptors regulate an impressive number of physiological functions including nerve activity, tension of smooth muscle, metabolism, rate and force of cardiac contraction, and the secretion of most glands in the body. Included among the ligands for GPCRs are

- Eicosanoids,
- Peptide hormones,
- Neurotransmitters - Acetyl choline,
- Lipid signaling molecules,

- Opioids,
- Biogenic amines - Nor epinephrine,
- Amino acids – GABA,
- Other peptide and protein ligands.

GPCRs are important regulators of nerve activity in the CNS and are the receptors for the neurotransmitters of the peripheral autonomic nervous system. For example, ACh released by the parasympathetic nervous system regulates the functions of glands and smooth muscle through its action on muscarinic receptors. NE released by the sympathetic nervous system interacts with adrenergic receptors to regulate cardiac function and the tone of vascular smooth muscle. Due to their number, physiological importance, GPCRs are targets for many drugs; almost half of non-antibiotic drugs act thro these receptors

Picture 1: Showing pathways of GPCR receptor activation and its effects



Source: Brunton LL, Chabner BA, Knollmann BC: *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th Edition:* www.accessmedicine.com

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ACTIVATION

When an agonist binds to a GPCR, there is a conformational change in the receptor that is transmitted from the ligand-binding pocket to the second and third intracellular loops of the receptor which couple to the G protein heterotrimer. This conformational change causes α subunit to exchange its bound GDP for GTP. Binding of GTP activates the α subunit and causes it to release both the βγ dimer and the receptor, and both the GTP-bound α subunit and the βγ heterodimer become active signaling molecules[90]. The interaction of the agonist-bound GPCR with the G protein is transient; following activation of one G protein, the receptor is freed to interact with other G proteins. Depending on the nature of the α subunit, the active, GTP-bound form binds to and regulates effectors such as adenylyl cyclase (via $G_s\alpha$) or phospholipase C (via $G_q\alpha$). The βγ subunit can regulate many effectors including ion channels and enzymes such as PI_3-K. The G protein remains dynamic until GTP bound to alpha subunit is hydrolyzed to GDP. The alpha subunit has a slow intrinsic rate of GTP hydrolysis that is modulated by a family of proteins termed *regulators of G protein signaling* (RGSs). The RGS proteins greatly accelerate the hydrolysis of GTP and are potentially attractive drug targets[91]. Once the GDP attached to alpha subunit hydrolyzed to GDP, the βγ subunit and receptor recombine to form the

inactive receptor-G protein heterotrimer basal complex that can be reactivated by another ligand-binding event.

DESENSITISATION

Receptors in addition to regulation of biochemical process & physiological function also subject themselves to many homeostatic and regulatory controls. These controls include

- Covalent modification
- Regulation of synthesis
- Association with other regulatory proteins
- Degradation of receptor and
- Relocalization within cell.

Similar regulation of transducer and effector protein occurs; through modulatory inputs from other receptors, and receptors always has the feedback regulation by their own signaling outputs.

Desensitization may be due to temporary inaccessibility of receptor to agonist and also from other receptors which are being synthesized and available on cell surface. Phosphorylation of G Protein Coupled receptors by specific G Protein Coupled receptor kinases (GRKs) has a main role in stimulating rapid desensitization. Phosphorylation of agonist-occupied GPCRs facilitates binding of cytosolic proteins like *arrestins* to receptor,

ends in uncoupling of G protein[92]. These β-arrestins stimulates proteins like PDE_4, which limit cAMP signaling which promote receptor sequestration from the membrane known as *internalization*, thereby, forming a scaffold that prevents further signaling steps.

CLINICAL FEATURES

Bronchial asthma, affects airways as an inflammatory disorder with chronic inflammation. There is no single pathognomic histological feature, there are common findings like cell infiltration with T-lymphocytes, neutrophils & eosinophils, epithelial cell dysplasia, typically with blocking of tiny bronchi with mucus; deposition of collagen below membrane; smooth muscle hypertrophy of cartilaginous tube; dropsical bronchi; activation of mast cell; and epithelial tissue removal in bronchi. Such inflammation is basis of chronicity and causes AHR & limitation of air flow[93].

Patients who are sensitive to allergens when exposed will result in inflammation of airway, AHR and symptoms. This may form as immediate response like wheeze or in four to six hours as late wheezing response.

Common allergens are

- Cockroaches

- House dust mites
- Cat dander and
- Seasonal pollens.

Considerably reducing such things decrease pathognomic findings and symptoms. Signs & symptoms differ wide according to patient. Bronchial asthma is presented by asthmatics that are episodic in nature, dyspnoea, tightness of chest & cough. Increased mucus secretion is commonly seen. Some asthmatics have irregular, occasional attacks whereas some patients have regular symptoms.

Signs of attack could occur itself or could also influence and increased by alternative factors as mentioned before. Signs are often severe at night; bronchiolar tone variation based on circadian rhythm and cartilaginous tube response is high between 3- 4 am, during which bronchial contraction is at its peak.

General findings raise the likelihood of acute attack. Mucosal edema, exaggerated secretions, and polyps in nose are usually present in allergic attack of asthma patients. Atopic dermatitis, eczema, or alternative presentation of dermatological disorders due to allergy may be present. Chest examination could also be seen without any signs between acute attacks in mild asthma. During very severe asthma attack, flow is also restricted so only diagnostic sign on auscultation will be widely decreased

breath sounds with increased time in expiration. Signs like hunching of shoulders &increased use in accessory muscles signify a increased labour of breathing.

LABORATORY FINDINGS

Arterial blood gas measurements is also traditional during a mild asthma attack exacerbation, however alkalosis and a rise within the alveolar-arterial oxygen distinction is prevalent. When there is very severe exacerbation, hypoxia develops & $PaCo_2$ comes back to normal. Hence mixture of accumulated acidosis & $PaCo_2$ could denote an imminent failure of respiration & also want for other means of ventilation like mechanical.

PULMONARY FUNCTION TEST

Physicians can determine obstruction in air flow on clinical examination; however there is need to assess severity and whether disease is reversible or irreversible. Analysis of disease ought to thus include spirometry (forced expiratory volume in one second [FEV_1], forced vital capacity, (FEV_1/FVC) both baseline & after starting short acting drugs. Also such investigation helps to confirm level of flow obstruction, also its reversibility. Airflow hindrance is shown by decreased FEV_1/FVC magnitude relation. Main result of obstruction of air flow shown through

rise of more than or equal to 12%, two hundred ml in FEV_1 or more than or equal to 15 % and 200 ml in Forced vital capacity[FVC] when using bronchodilator of minimal action. An affirmative drug action powerfully proves designation of attack however scarcity of positive response within the PFT doesn't confirm prognosis during a clinical study about bronchodilator. High flow hindrance ends up with more air trappings, a rise in respiratory volume and subsequent decrease in restrictive ventilatory deficiency like restrictive lung disease.

Bronchial stimulation testing using methacholine or histamine in inhaled form is also helpful once you suspected as asthmatic, however PFT is not a diagnostic. Bronchial stimulation isn't counseled when FEV_1 is a smaller amount than Sixty five% of the expected.

An affirmative test using methacholine is outlined as ≥ twenty percent decrease within Forced expiratory volume1 at reaction to strength of eight mg/ml or low. Challenge test using exercise is also helpful in asthmatics having exercise stimulated bronchospasm.

PEFR meters are hand-held instruments planned as watching tools used personally. Monitoring of PEFR will prove variability in peak flow; assess severity of asthma attack, and supply patient & practitioner with readings to decide basic treatment. Also different opinions on whether or not using PEFR has effect on prognosis; however PEFR is suggested as it

may assist to make sure final diagnosis, to enhance management for patients with poor flow in airways, and to know environmental, occupational reasons for signs and symptoms. Foreseen measurement of PEF differ depending upon gender, age etc

Comparing with standard values is of less value than comparing patient's baseline values .PEF varies diurnally, low on morning and high at mid day. Peak Expiratory flow ought to be seen by early morning before giving drug and within noon after drug. A twenty percent diurnal change or day change indicates poorly controlled disease. Peak expiratory flow rate below two hundred ml/min shows very severe air flow obstruction.

OTHER INVESTIGATIONS

Regular X-ray of chest in cases with regular attack is typically traditional else has solely increased inflation. Alternative signs could embrace cartilaginous tube wall edema and decreased lung shadows. X ray chest is needed once respiratory illness, another any other disease simulating asthma or consequence of disease like pneumothorax or any other is suspected.

Intradermal skin test to know sensitivity to allergens in environment will determine type I allergic reaction in cases with regular

attack, could have the gain of treatment focused at allergy sensitivity. Investigation for GERD ought to be thought in BA patients having persistent signs & symptoms and recurrence. Noninvasive investigation for airway inflammation includes measuring symptom evoked mucus, or fractional NO concentration in expired breath (FeNO), gives positive outlook on diagnosis & management methods. Managing corticosteroid dosage to reduce mucus symptom seems to scale back the recurrence of exacerbations in comparison to typical management, however information are differing concerning the importance of FeNO on asthma outcomes.

DIFFERENTIAL DIAGNOSIS

It's important to think about situations that simulate respiratory disease in cases with atypical signs & symptoms or decreased response to medical aid. Such disease usually make up in any of 4 types:

- Psychiatrical disorders.
- Upper airway disorders
- General vasculitides, and
- Lower airway disorders

Upper respiratory tract diseases that simulate BA are

- Vocal cord palsy

- Inhalation injury
- Foreign body aspiration
- Tracheal narrowing
- Vocal cord dysfunction syndrome
- Laryngotracheal masses,
- Tracheomalacia
- Airway swelling.

Lower respiratory diseases are

- Chronic bronchitis
- Non-asthmatic COPD
- Emphysema
- Cystic fibrosis
- Allergic bronchopulmonary fungal infection
- Bronchiectasis
- Eosinophilic pneumonia
- Bronchiolitis obliterans.

Systemic

- Churg-Strauss syndrome.

Psychiatric causes like

- Conversion disorders,
- Vocal fold dysfunction
- Episodic laryngeal dyskinesis.
- Emotional laryngeal asthmatic
- Münchausen syndrome

CURRENT TREATMENT

The goals of bronchial asthma medical aids is mainly attenuate symptoms of disturbing the regular activity (also exercise), also to reduce recurrent attacks, and cut back or reduce necessity for acute visits to hospitals, and take care of normal pulmonary function.

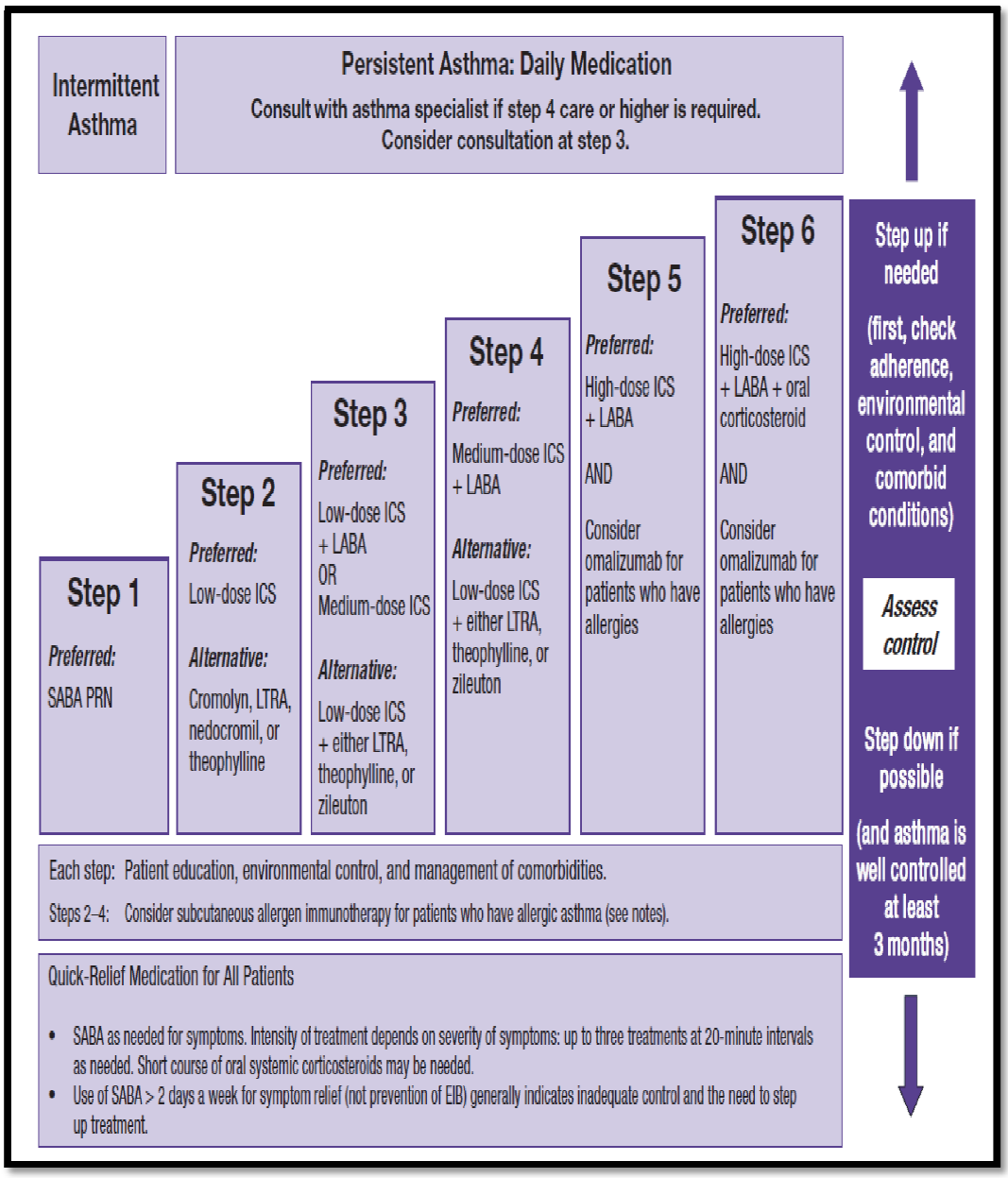
Such aim ought to be seen whereas giving drug treatment with low side effects and fulfilling the expectation of cases and their relations. Recommendation by NAEPP 3 points regular treatment with anti-inflammatory property like corticosteroids in inhaled route as main point of management of severe bronchial asthma.

The management includes chronic controller medication and short term reliever medications. Long run medications embrace anti-inflammatory medication like corticosteroids, long acting bronchodilators, anti cholinergic, phophodiesterase inhibitors, leukotriene

modifiers, desensitisation with single allergen therapy, omalizumab and vaccination.

Short acting reliever medication includes beta agonist, high dose corticosteroids and antimicrobials with respiratory support if necessary.

Picture 2 :Showing Stepwise treatment of Asthma



DRUGS REDUCING INFLAMMATION

Steroids are the foremost active and systematically acting drug presently given. These drugs cut back acute & chronic inflammation, leading to less respiratory disease signs, increase in flow of air, attenuate airway hyperresponsiveness, and decreased asthma recurrences. Such drugs additionally enhance the effect of beta agonists.

Corticosteroids in inhalation route are most well-liked, initial drugs for cases with chronic respiratory disease. Such drugs fasten the recovery of airflow hindrance & reduce relapse. Patients with regular symptoms or recurrences who are not on inhaled steroids ought to be started with the same. Foremost choice of drug and required dosing depends upon status of patient & response.

Dose depends on type of drug and device used, most patients required management given two times daily for good management of disease. Some patients it's fine with daily dosing. Systemic side effects might happen with large dose inhaled steroid hormone treatment. Local side effects includes irritation and fungal infection which can be overcome by washing mouth after MDI use and this also prevents systemic absorption of the drug.

Systemic steroids are effective primary treatment in cases with severe BA and for patients with recurrences, those do not respond to treatment quickly to inhalation therapy. In such patients there is need of chronic suppression of signs, many efforts have done to reduce the dosage to less amount required to manage the symptoms Day after day treatment is most well-liked. Coinciding management along with calcium & Vit D should be started to forestall osteoporosis due to corticosteroid in chronic administration. Bisphosphonates might provide further protection for those patients. Speedy discontinuance of systemic route of steroids once in chronic use might cause adrenal insufficiency.

Delay in administering corticosteroids might lead to late benefits due to these drugs. So, oral steroids should be there for quick treatment at home for several patients with wide range of respiratory disease. Borderline dose of systemic steroids for such is not known. Patient prednisolone “burst” treatment is 0.5–1 mg/kg per kg per day as single or 2 divided doses for three to ten days.

Severe manifestation need admission in hospital usually need one mg per kg of prednisolone or methylprednisone every sixth hour for forty eight hours or when FEV_1 comes back to fifty percent of baseline value. Dosage is then given up to sixty mg per day until PEFR is seventy percent of expected value, there is no advantage when dose is increased

in severe manifestations. Also intravenous route will be better for patients who are critically ill to avoid first pass metabolism.

BETA- ADRENERGIC AGONISTS

Beta agonists both long acting and short acting are available. Long acting drugs offer bronchodilatation for up to twelve hours, when one dose is administered. Commonly used long acting agonists include salmeterol and formoterol. They are available as dry powder and are administered using delivery devices. These drugs are indicated for control of asthma symptoms, night symptoms and for bronchospasm induced by exercise in a long run. These drugs on addition to ICS they produce the management comparable to the one which is achieved on doubling the dose of ICS. These long acting drugs are not used as monotherapy, as they lack anti inflammatory property. Additionally in two large trials when they are given as monotherapy it lead to risk of severe and fatal asthma attacks. This risk could have been even due to genetic variations in the beta adrenergic receptors or some other individual variation and hence this remains as a locality of controversy. The effectiveness of LABA and ICS has led to selling of them in combination medication that delivers each agent at the same time. Formoterol and budesonide have shown effectiveness even as both maintenance and rescue medication owing to the short time of onset for formoterol.

Though exacerbation short acting drug are the most effective, it includes albuterol, levoalbuterol, terbutaline, pirbuterol etc. It is the drug of choice and most patients ought to take one among these agents during acute symptoms. There are no big studies stating one drug is better over other. These drugs relax the airways by relaxing the smooth muscle and by increasing the airflow thereby relieving the broncho constriction and reducing the symptoms. To prevent exercise induced bronchospasm they are administered before exercise. Selective beta 2 agents have less cardiac receptor stimulation compared to non selective group of beta agonist. Inhaled β-adrenergic medical aid is as effective as oral or parenteral therapy in airway smooth muscle and improving acute respiratory disease and offers the benefits of speedy onset of action (< five minutes) with fewer systemic side effects. Repetitive administration produces progressive bronchodilatation. One or 2 inhalations of a short-acting inhaled β_2-agonist from MDI are sometimes enough for mild to moderate symptoms. Severe exacerbations often need higher doses: 6–12 puffs each 30–60 minutes of bronchodilator by MDI with an inhalation chamber or 2.5 mg by nebulizer offer equivalent bronchodilation.

Administration by nebulization doesn't provide more effective delivery than MDIs however will offer higher doses. There is a difference in dosing between the metered dose inhaler and nebulizer. Without the

note on dose nebulizer are advised for patients who are not able to co ordinate between drug delivery and inhalation.

ANTI-CHOLINERGICS

The long-acting anti-cholinergic tiotropium has been studied as add-on medical aid for patients who have either a bronchodilator response or a positive methacholine challenge that's not adequately controlled with low-dose ICS. With 14 weeks of treatment, adding tiotropium resulted in enhancements in PEF, FEV_1, and symptom control; the enhancements were larger than those achieved by doubling the dose of the ICS for an equivalent amount of time. The addition of tiotropium wasn't inferior to the addition of salmeterol. The short treatment amount doesn't allow a determination of whether or not long β_2-agonists are similar to tiotropium in reducing asthma exacerbations and whether or not it's a good and safe alternative to long β2-agonists for the long-term management of asthma.

Anti-cholinergic agents reverse vagus mediation of bronchospasm however allergen mediated or exercise evoked spasm is not reversed. They'll diminish mucous gland secretion present in respiratory disease. Ipratropium bromide, a quaternary by-product of atropine freed from atropine like side effects, is a smaller amount effective than β2-agonists for relief of acute spasm, however it's the inhaled drug of choice for patients with intolerance to β2-agonists and with bronchospasm as a

result of b-blocker medications. Ipratropium bromide reduces the rate of hospital admissions once additional to inhaled short-acting β2-agonists in patients with moderate to severe asthma exacerbations.

PHOSPHODIESTERASE INHIBITORS

Theophylline provides mild bronchodilation in wheezy patients. Theophylline also mediates anti-inflammation property, immunomodulation properties, augments mucociliary drainage, and strengthens diaphragmatic contraction. Extended release theophylline preparations are useful in controlling night symptoms and as additional therapy in patients with severe type of asthma attack whose symptoms are inadequately controlled by ICS. Once added to inhaled corticosteroids, theophylline could permit equivalent control at lower corticosteroid doses. Theophylline serum concentrations have to be compelled to be monitored closely as a result of very small therapeutic index, variations in drug metabolism, and factors affecting absorption and metabolism.

At therapeutic doses the adverse effects includes sleep disorder, provocation of indigestion and GERD symptoms, and urinary flow obstruction with benign prostatic hypertrophy. Toxic dose side effects embrace nausea, vomiting, increased heart rate, headache, epilepsy, hyperglycaemia, and electrolyte imbalance. New drugs are in clinical trials for selective inhibition of PDE-4.

Methylxanthines don't seem to be recommended for medical care during acute attacks. Aminophylline as a single drug therapy is found to be inferior to beta agonism for acute attack. And if patients who are on theophylline has an attack and acute doses of theophylline are planned to be used as management, then their serum concentrations are to be measured before administration to prevent toxicity.

LEUKOTRIENE MODIFIERS

Leukotrienes are the mediators of airway obstruction through smooth muscle contraction, augmenting vascular permeability and mucous secretion, accompanied with activation of inflammatory cells. Drugs modifying leukotriene includes 5-lipoxygenase inhibitors, Zileuton which decreases the production of leukotrienes, and drugs antagonizing the leukotriene receptor includes montelukast and zafirleukast. In randomised controlled trials, these agents showed improvements in PFT and decreased the dose of beta agonist therapy when used as rescue medication. These are the drug of choice in mild types of asthma, but as a monotherapy they are inferior to inhaled corticosteroids. In pragmatic, real-life community trials, leukotriene receptor antagonists were equivalent in efficaciousness to an inhaled corticosteroid as first-line long-term controller medication or to a long-acting β-agonist as add-on medical care. Zileuton will cause raise in LFT which is reversible on

stoppage of drug and few people with Churg-Strauss syndrome has been diagnosed in an exceedingly tiny range of patients on chronic intake of montelukast or zafirleukast, though this can be suspected to be an impact of corticosteroid withdrawal as against an immediate drug effect.

DESENSITIZATION

Immunotherapy for specific allergens is also thought of in hand-picked asthma patients who have exacerbations of asthma symptoms when exposed specifically to the specific allergen and who are not responding to the conventional treatments.

Studies show a reduction in respiratory disease symptoms in patients treated with single-allergen immunotherapy. Due to the chance of immunotherapy-induced bronchoconstriction, it ought to be administered only in a setting wherever such complications are often at once treated.

OMALIZUMAB

Omalizumab is an IgE antibody produced by recombinant technology and acts by binding to the immunoglobin without mast cell activation. Omalizumab has proved to reduce the dose of corticosteroid use when administered to patient with moderate to severe type of asthma.

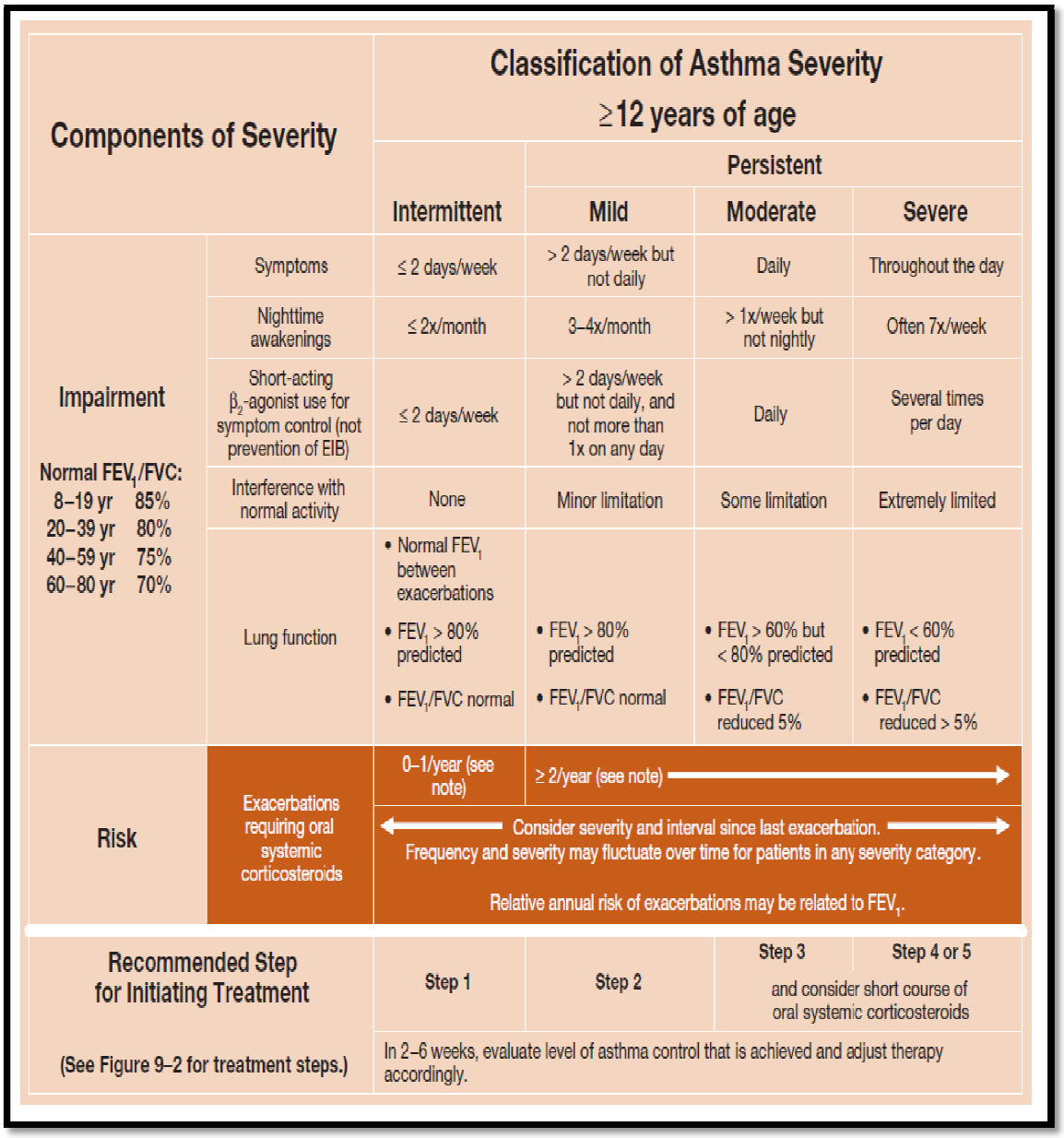
VACCINATION

Patients with respiratory disease ought to receive pneumococcal vaccination and annual asthma influenza vaccine. Out of active and inactive vaccine, inactive vaccines are related to lesser side effects; however use of the live attenuated influenza vaccine intranasally could also be related to a rise in asthma exacerbations in young children.

ANTIMICROBIALS

Various studies have demonstrated that infection with viruses especially with rhinovirus and bacteria's like mycoplasma or chlamydia may lead to acute exacerbation. Hence usages of antibiotics are suggested during these exacerbations. Regular usage of antibiotics without exacerbation is not recommended, as there are no consistent testimonies which claim to improve clinical outcome with antibiotic usage. Antibiotics ought to be thought of once who are at high probability for respiratory tract infections like fever or evidence of pneumonia and sinusitis.

Picture 3: Illustrating classification of severity of asthma



Components of Severity		Classification of Asthma Severity ≥12 years of age			
			Persistent		
		Intermittent	Mild	Moderate	Severe
Impairment Normal FEV_1/FVC: 8–19 yr 85% 20–39 yr 80% 40–59 yr 75% 60–80 yr 70%	Symptoms	≤ 2 days/week	> 2 days/week but not daily	Daily	Throughout the day
	Nighttime awakenings	≤ 2x/month	3–4x/month	> 1x/week but not nightly	Often 7x/week
	Short-acting β_2-agonist use for symptom control (not prevention of EIB)	≤ 2 days/week	> 2 days/week but not daily, and not more than 1x on any day	Daily	Several times per day
	Interference with normal activity	None	Minor limitation	Some limitation	Extremely limited
	Lung function	• Normal FEV_1 between exacerbations • FEV_1 > 80% predicted • FEV_1/FVC normal	• FEV_1 > 80% predicted • FEV_1/FVC normal	• FEV_1 > 60% but < 80% predicted • FEV_1/FVC reduced 5%	• FEV_1 < 60% predicted • FEV_1/FVC reduced > 5%
Risk	Exacerbations requiring oral systemic corticosteroids	0–1/year (see note)	≥ 2/year (see note) →		
		← Consider severity and interval since last exacerbation. → Frequency and severity may fluctuate over time for patients in any severity category. Relative annual risk of exacerbations may be related to FEV_1.			
Recommended Step for Initiating Treatment		Step 1	Step 2	Step 3 and consider short course of oral systemic corticosteroids	Step 4 or 5 and consider short course of oral systemic corticosteroids
(See Figure 9–2 for treatment steps.)		In 2–6 weeks, evaluate level of asthma control that is achieved and adjust therapy accordingly.			

Picture 4: Illustrating classification of asthma control

Components of Control		Classification of Asthma Control (≥ 12 years of age)		
		Well Controlled	Not Well Controlled	Very Poorly Controlled
Impairment	Symptoms	≤ 2 days/week	> 2 days/week	Throughout the day
	Nighttime awakenings	≤ 2x/month	1-3x/week	≥ 4x/week
	Interference with normal activity	None	Some limitation	Extremely limited
	Short-acting β_2-agonist use for symptom control (not prevention of EIB)	≤ 2 days/week	> 2 days/week	Several times/day
	FEV_1 or peak flow	> 80% predicted/personal best	60-80% predicted/personal best	< 60% predicted/personal best
	Validated questionnaires			
	ATAQ	0	1-2	3-4
	ACQ	≤ 0.75[1]	≥ 1.5	N/A
	ACT	≥ 20	16-19	≤ 15
Risk	Exacerbations requiring oral system corticosteroids	0-1/year	≥ 2/year (see note)	
		Consider severity and interval since last exacerbation		
	Progressive loss of lung function	Evaluation requires long-term follow-up care		
	Treatment-related adverse effects	Medication side effects can vary in intensity from none to very troublesome and worrisome. The level of intensity does not correlate to specific levels of control but should be considered in the overall assessment of risk.		
Recommended Action for Treatment (see Figure 9-2 for steps)		• Maintain current step • Regular follow-ups every 1-6 months to maintain control. • Consider step down if well controlled for at least 3 months.	• Step up 1 step and • Reevaluate in 2-6 weeks. • For side effects, consider alternative treatment options.	• Consider short course of oral systemic corticosteroids, • Step up 1-2 steps, and • Reevaluate in 2 weeks. • For side effects, consider alternative treatment options.

TOOL USED

Various measures for control of asthma

There are numerous strategies used to evaluate the asthma control. It includes ACSS (asthma control scoring system), ACT (asthma control test), ACQ (asthma control Questionnaire), ATAQ (Asthma therapy assessment questionnaire), AQLQ (Asthma Quality of life questionnaire) and many more.

Among these ACT and ACQ are the foremost often used tests. The easy to administer questionnaires include ACT and ATAQ as they're symptom based evaluation, whereas ACQ and ACSS include pulmonary function test. Additionally limitation of ATAQ is that it doesn't include the presence of day time symptoms. AQLQ is extremely helpful for quantification of quality of life in bronchial asthma and response treatment than the control. ACT in specific has been found to be helpful for primary health care development in developing countries[94].

LITERATURE FOR ASTHMA CONTROL TEST

Accurate assessment of control is a very important a part of asthma management. Inadequate assessment of control could be a major issue for poor control of asthma[95]. It is not patients alone who overestimate their control[96, 97], clinicians conjointly tend to try to do the same[98,99,100].Asthma

control test (ACT) is a self-administered tool for assessing patients with poor control, comprising five-item questionnaire with symptoms and daily activites are recalled for 4 weeks , need of rescue medication, and self-rating of asthma control[101]. It has been made known to show a relationship with a physician's ratings of control[102] .5-point scale (for symptoms and activities: 1=all the time to 5= not at all). The scores vary from 5 (poor control of asthma) to 25 (complete control of asthma), with higher scores reflective greater asthma management. An ACT score >19 indicates well-controlled asthma. The only limitation of this form is, it's a multidimensional construct, but doesn't include objective measures of airway caliber. ACT has conjointly been validated to be used by mail[103] and by telephone[104]. Be aware of asthma control as the main intension for treatment, is the revised guidelines of GINA in 2006 for prompt management, supported based on control instead of on disease severity[105]. Same concept is also seen with different national guidelines[106, 107].

The modified GINA guideline defines 3 levels of control; 'Well-controlled', 'partly controlled' and 'uncontrolled'. While the GINA criteria define a conceptual framework for assessing asthma control, the ACT could be a sensible tool enabling clinicians to use the GINA recommendations. The ACT is straightforward to use and provides a

consistent, numeric score which will assist in watching symptom control over time[102]. Wider use of the ACT and similar instruments ought to facilitate assessment of the asthma burden in the community. Better assessment of control could lead to improved management, greater patient awareness and understanding of their asthma, and ultimately to better patient outcomes.

METHODOLOGY

STUDY CENTRE:

The study was done in the Department of Pharmacology, PSG Institute of Medical Sciences and Research and the Department of Endocrinology, PSG Hospitals, in collaboration with the PSG Centre for Molecular Medicine and Therapeutics (CMMT).

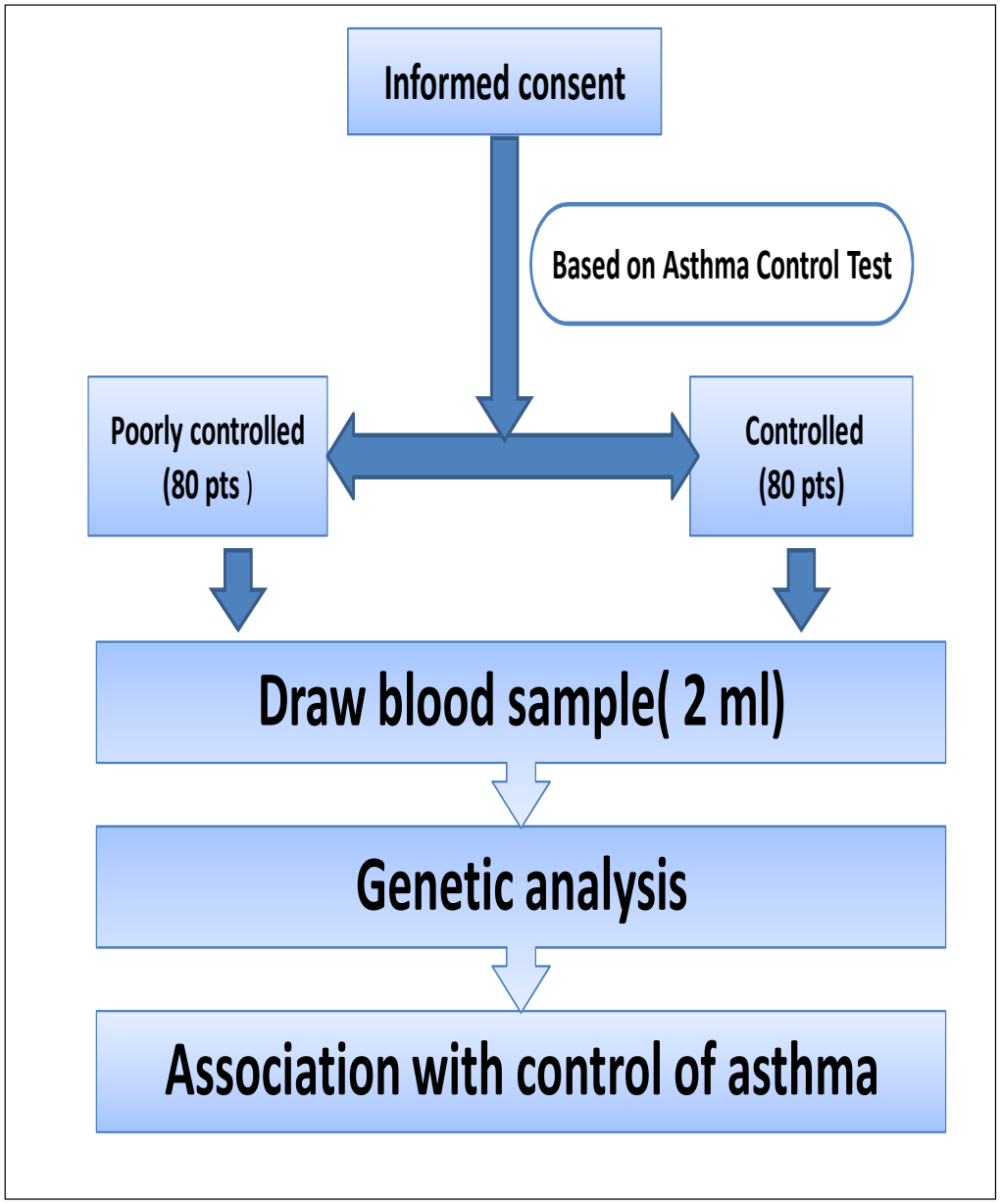
STUDY SUBJECTS:

Inpatients and outpatients of Respiratory Medicine and Pulmonology department with clinical diagnosis of asthma from February 2013 to July 2014

STUDY DESIGN:

The study was a cross sectional study .After obtaining informed consent and fulfilling the inclusion exclusion criteria patients were given the self administered questionnaire in their language and divided into well controlled and poorly controlled group. Blood sample was collected from all the patients and their genetic analysis was done.

Picture 5 :FLOWCHART OF STUDY DESIGN



SAMPLE SIZE:

Sample size of 160 patients were recruited comprising of 80 patients in each group

ETHICAL APPROVAL:

The study protocol was approved by the Institutional Human Ethics Committee (IHEC) preceding the start of the study. The details and the purpose of the study protocol were explained to each participant individually and their doubts were clarified before obtaining informed consent. The informed consents forms provided to the participants were either in English or in Tamil. The participants, who gave written informed consent, and came under the inclusion criteria, were enrolled for the study. A copy of the consent form is attached in the annexure.

TOOL USED

Asthma control test -a validated self administered questionnaire used to assess control of asthma in patients.

Score >19 – well controlled Score ≤ 19 – poorly controlled

INCLUSION CRITERIA

- All patients diagnosed to have asthma according to the accepted guidelines and on treatment atleast for past one year.
- Ability and willingness to provide informed consent
- Age >12 years

EXCLUSION CRITERIA

Nil

GENETIC ANALYSIS:

Steps involved in genotype analysis were;

- DNA extraction
- PCR to amplify GRK 5 gene
- RFLP using BsrI restriction enzyme
- PAG electrophoresis of the digested product

DNA EXTRACTION

CELL LYSIS

- Blood (300µl) + I volume of cell lysis buffer (buffer c) + 3 volume of water, mix up by inverting the samples.
- Incubate the samples on the ice (refrigerator) for 10 minutes
- Centrifuge the samples at 4° C for 20 minutes at 4000 rpm

- Discard the supernatant and leave the cuvettes open for a while.
- And add 150µl of ice cold buffer in every sample.
- Again add 480 µl of autoclaved H_2O and close the cuvette.
- Disturb the pellet by using vortexer or by mixing with pipette.
- Centrifuge the sample at 4°C for 30 minutes at 4000 rpm.
- Yet again, discard the supernatant.

NUCLEIC ACID LYSIS

- As soon as supernatant discarded, add 720µl of nucleic acid lysis buffer , along with add 12-15µl of RNase A
- Vortex the samples and incubate it for 10 -15 minutes at 37°C.
- Add 30µl of 10% SDS and 35 µl of proteinase K
- Incubate the sample in water bath at 55°celcius for two-three hours.
- Add phenol: chloroform (1:1), vortex the samples and centrifuge for ten minutes at15800rpm.
- Extract the supernatant of the samples (aqueous layer) and transfer it to another tube.
- Same volume of chloroform is added, vortex the samples and centrifugefor ten minutes at 15800 rpm .
- Transfer the supernatant or the aqueous layer to another tube, add 0.1× volume of 3M of NaaC (90) at ph of 6.0
- Again, add 2× volume of 100% ethanol.

- Store the samples overnight at -20°Celcius. (10 mins-70*)
- DNA can be found at the pellet after centrifuging at 15800 rpm for 20 minutes.
- Completely wash the DNA wit h 70 % of ethanol dry it and re suspend the DNA pellet in 50µl TE buffer.

AGAROSE GEL ELECTROPHORESIS

Reagents required:

1. TAE buffer 50 X stock:

   24.2g Tris base

   5.7ml Glacial Acetic acid

   10ml 0.5M EDTA pH 8.0

   Dissolve in 100 ml Distilled water

2. TAE buffer 1X Tank buffer:

   Dilute the 50X TAE buffer to 1X by adding water. For eg (10ml 50X TAE + 490ml Water) it becomes 1X TAE. Use it for electrophoresis tank to run the gel.

3. DNA loading dye
4. Ethidium bromide.
5. Agarose powder.

Gel preparation:

- Take fresh 50ml 1X TAE buffer (based on gel template size) in a conical flask and add 0.4g agarose (0.8% gel), heat the solution well.
- Before that seal the Gel template with cello tape and keep the well template ready.
- Then, add 2ul ETBR solution with heated 5oml solution mix well and pour it in sealed gel template and leave it for a while to become a gel.

Electrophoresis:

- Load the 1X TAE buffer in tank (~600ml required) and keep the gel plate inside the tank.
- Then take 2ul DNA loading dye and 8ul DNA (isolated) mix well and load it in wells carefully.
- Set the volt at 75V and run it for 30-45 mins. Then analyze the DNA bands using gel document machine.
- Quantification of the extracted genomic DNA was done using Nanodrop quantification after 0.8% agarose gel electrophoresis.

GRK 5 RFLP PCR

REQUIREMENTS:

1. **PRIMERS (SIGMA)**

S. NO	PRIMERS	2^0 STOCK CON	FINAL CON
1	Forward primer GRK5 bsrF	1µM	50 nM
2	Reverse primer GRK5 bsrR	1µM	50 nM

2. **DNTPs (Himedia)**

S. NO	DNTPS STOCK	1^0STOCK CON	2^0 STOCK CON	FINAL CON
1	100Mm each DNTPS	10mM each DNTPS	2.5mM each DNTPS	100µM each DNTPS

3. **Taq DNA polymerase** (colour Taq Genei) (Stock 1 Unit/ µl, Final con 0.1 unit)
4. **Taq Buffer A** (Genei) (Stock 15mM $Mgcl_2$, Final con 1.5mM $Mgcl_2$)
5. **DNA Sample** Optimised concentration: 0.03µg/ 20µL
6. **Milliq water**

PCR REACTION MIX

S. NO	COMPONENTS	VOLUME	FINAL CONCENTRATION
1	Forward primers 1 GRK5 bsrF - 1 µM	1 µL	50 nM
2	Reverse primers 1 GRK5 bsrR - 1 µM	1 µL	50 nM
3	Taq Buffer A	2 µL	1.5mM $Mgcl_2$
4	DNTPs	0.8 µL	100µM each DNTPS
5	Taq enzyme	0.2 µL	0.01 unit
6	DNA sample (0.03µg)	-	0.03µg/ 20µL
7	Milli Q	UPTO 20 µL	

PCR PROGRAME

1. **Initial denaturation - 94^{0}C for 10 min**
2. **Denaturation - 94^{0}C for 30 sec** } **40 CYCLES**
3. **Annealing - 60^{0}C for 30 sec**
4. **Extension - 72^{0}C for 1 min**
5. **Final extension - 72^{0}C for 5 min**
6. **Then held at 4^{0}C**

- PCR amplification confirmed on 2% agarose gel
- PCR product size is **224 bp**

RESTRICTION DIGESTION

REQUIREMENTS

1. BstUI/ Bsh 1236 I (Fermentas 10units/ µL)
2. 10 x Buffer R
3. PCR product

Optimized Concentration

For wild Homozygous - 2 µg

For Mutant Homozygous - 2 µg

For Heterozygous - 3 µg

4. 37^{o}C & 65^{o}C incubator

REACTION MIX FOR RFLP

S. NO	COMPONENTS	VOLUME
1	BsrI Enzyme	1 µl
2	10X Buffer	5 µl
3	DNA concentration	1 µg
4	Total Rxn volume	50 µl

- Mix gently & spin down for few seconds
- Incubate at 65^{0}C for 15-40 mins
- Restriction digestion product is inactivated at 80^{0}C for 20 min before gel electrophoresis

POLYACRYLAMIDE GEL ELECTROPHORESIS

- The DNA Fragments separated using 15 % Polyacrylamide gel electrophoresis
- Polyacylamide gel prepared from the following reaction mix

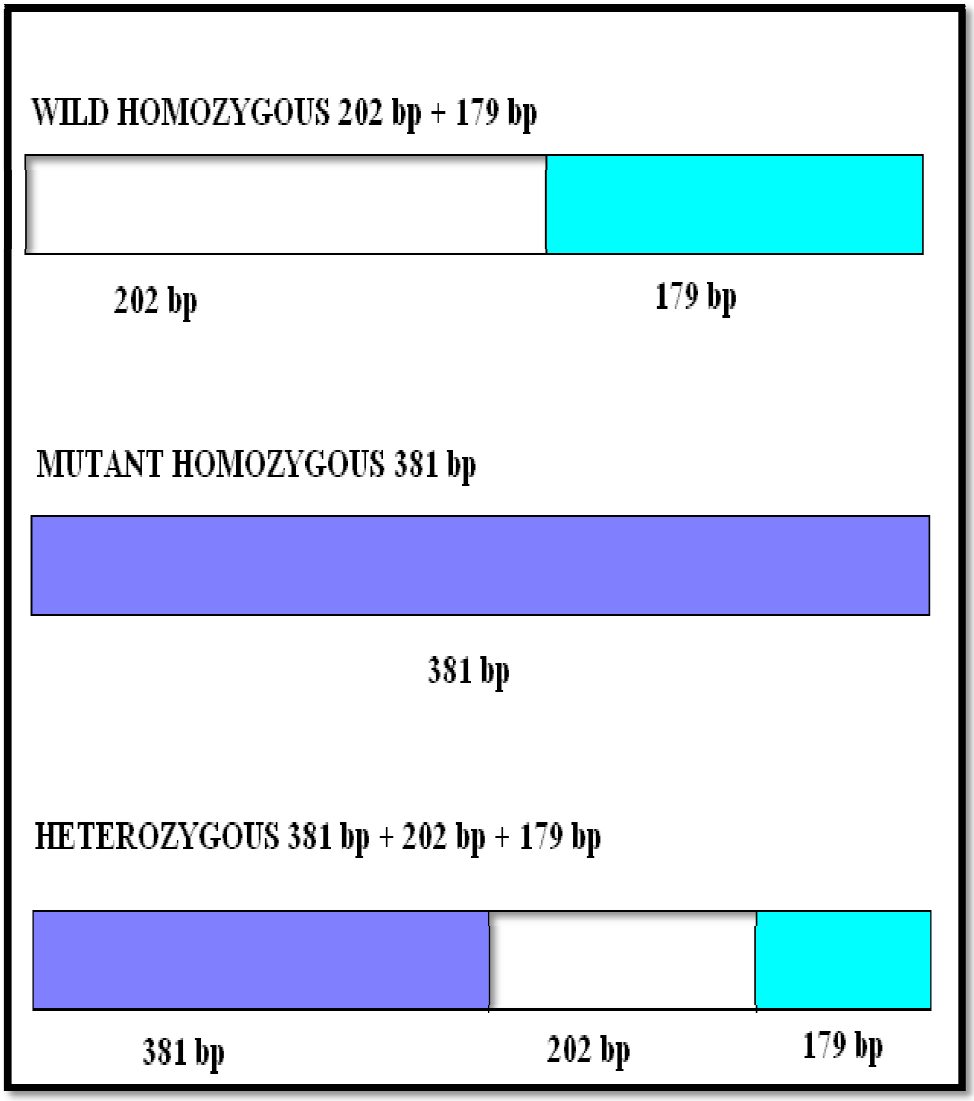
15% GEL	5mL	10mL	15mL	20mL	25mL	30mL	40mL	50mL
Water	1.2	2.3	3.5	4.6	5.7	6.9	9.2	11.4
A:B (30:0.8)	2.5	5	7.5	10	12.5	15	20	25
1.5M Tris pH 8.8	1.3	2.5	3.8	5	6.3	7.5	10	12.5
10% SDS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.020

- Polyacylamide gel was run in Amersham electrophoresis system at 100 V for about 5 hours
- The gel was then stained with ethydium bromide
- The stained gel was viewed in a Chemiluminescence gel documentation system to identify the DNA fragments

IDENTIFICATION OF GENOTYPES

- Restriction occurs when '**A**' is present ie (Homozygous) Wild type thus produce 202 bp + 179 bp
- Restriction does not occur when '**T**' is present ie (Homozygous) Mutant type thus produce only 381 bp
- Restriction occurs when '**A**' & '**T**' both are present ie (Heterozygous)Wild type thus produce 381 bp + 202 bp + 179 bp

Picture 6 : Showing the Base pair size of the GRK 5 gene



RESULTS

Fig 1 : Distribution of Male and Female among asthmatics

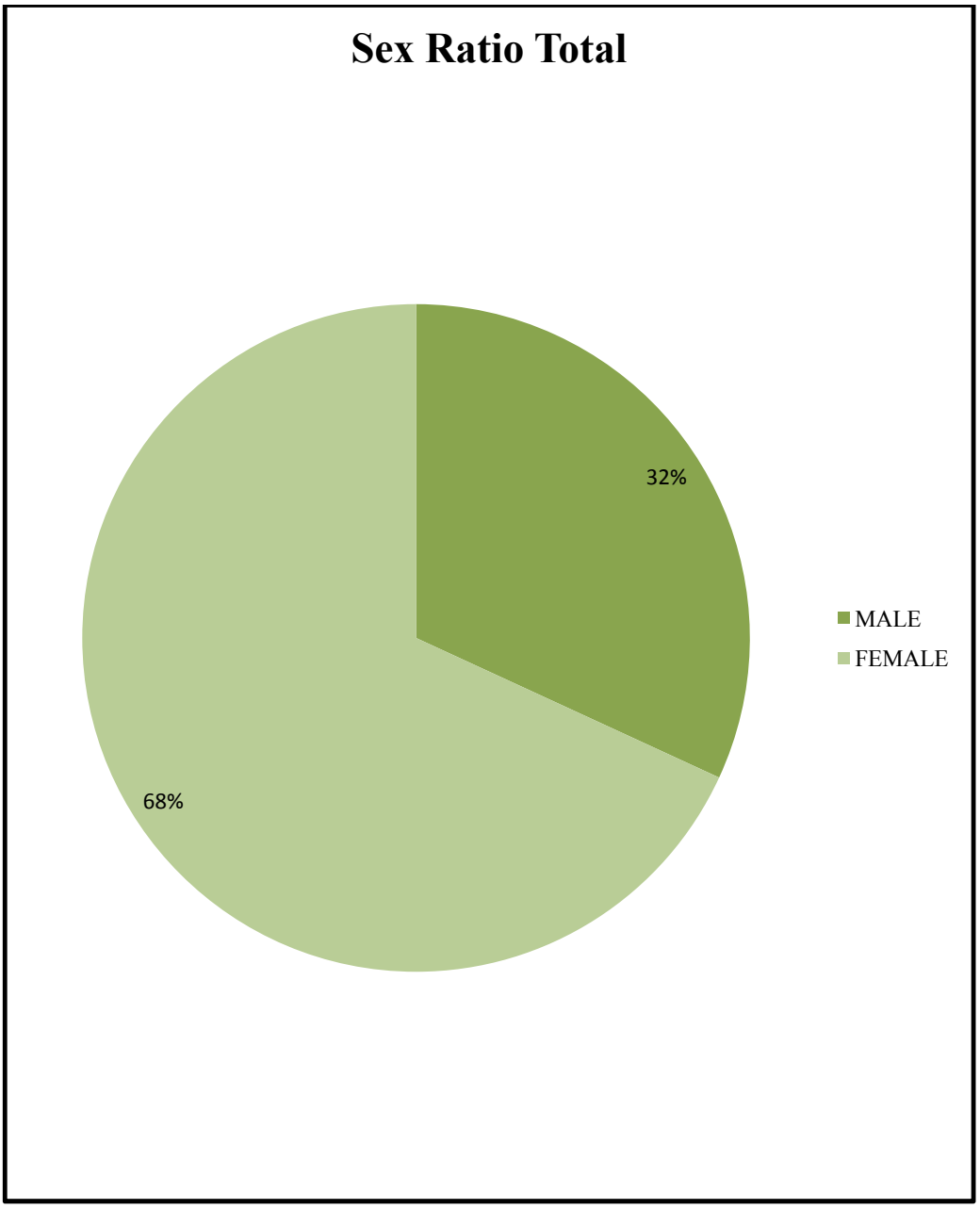


Fig 2 : Distribution of Male and Female among well controlled and poorly controlled asthmatics

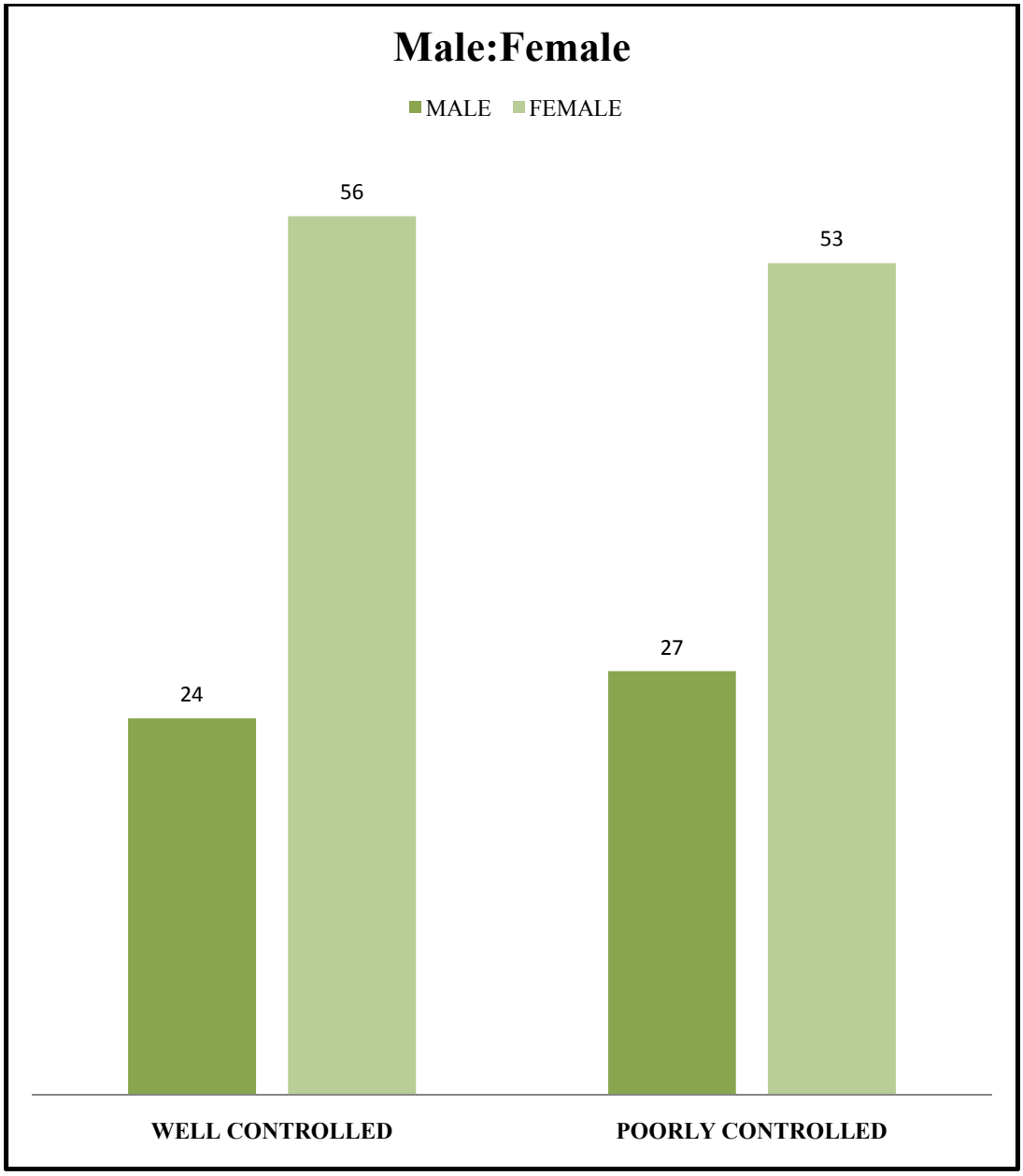


Fig 3 : Distribution of Well Controlled, Poorly controlled patients in reference to the age

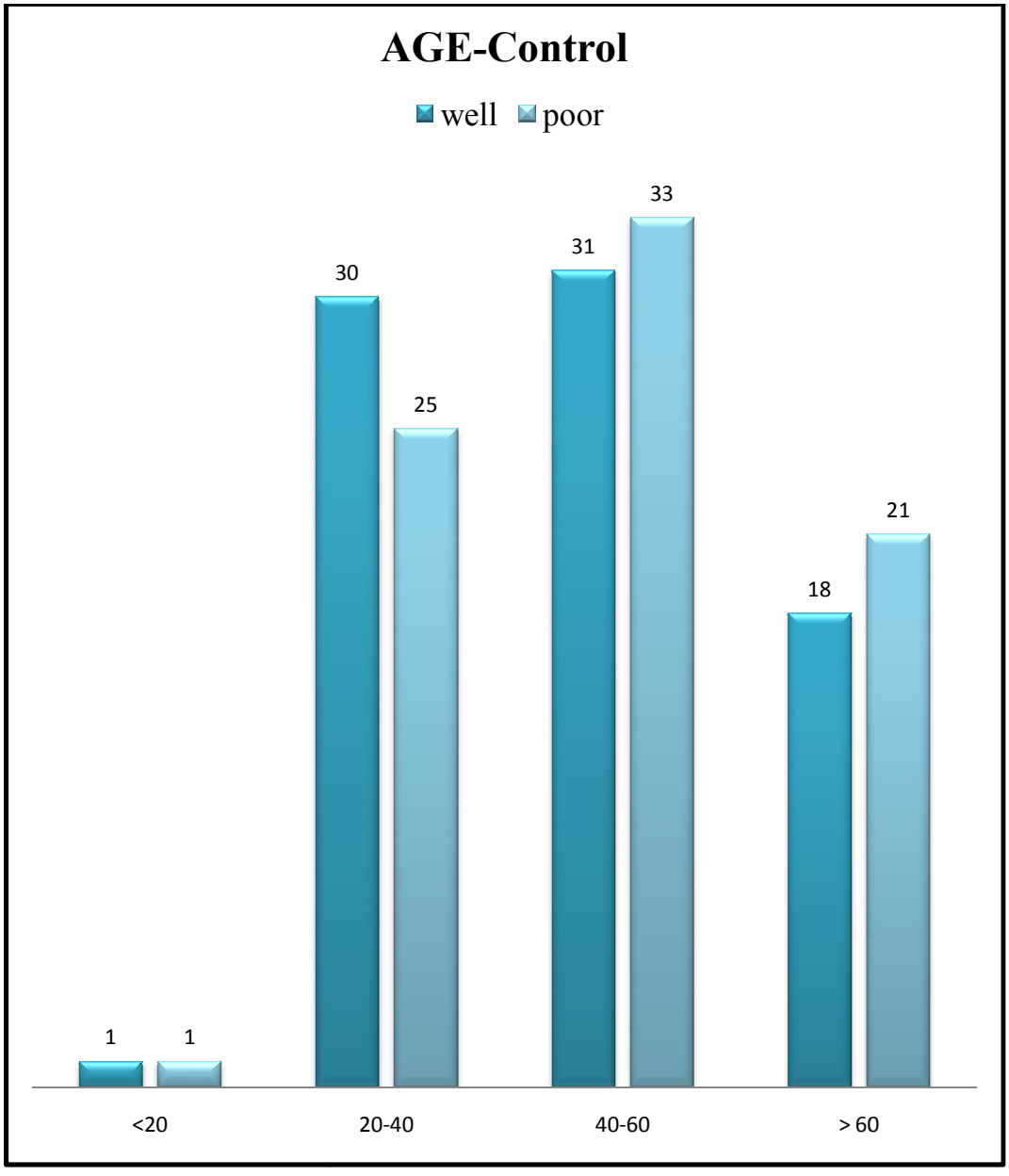


Fig 4 : Distribution of Well controlled, Poorly controlled patients according to BMI

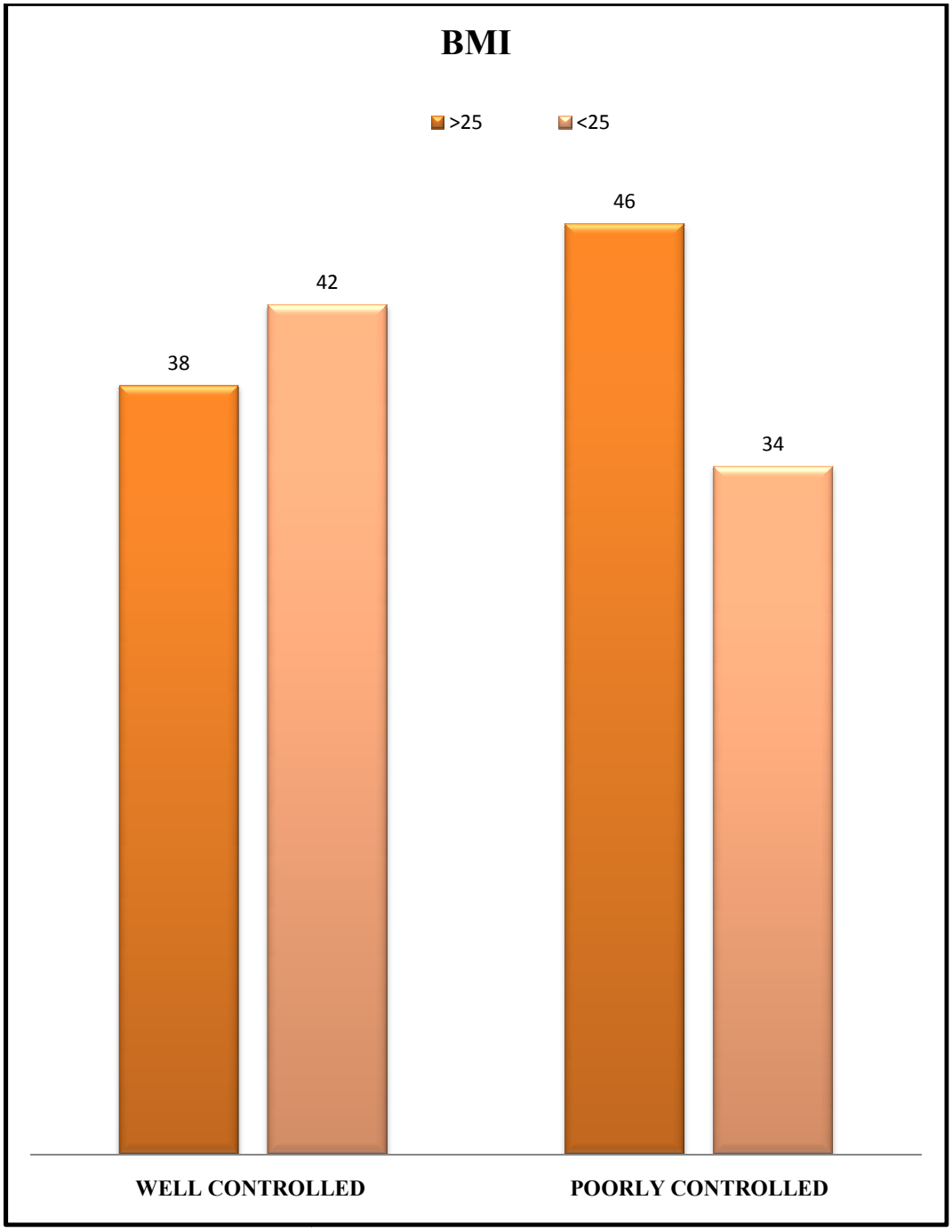


Fig 5 : Distribution of Well and poorly Controlled patients with H/O environmental hazard exposure

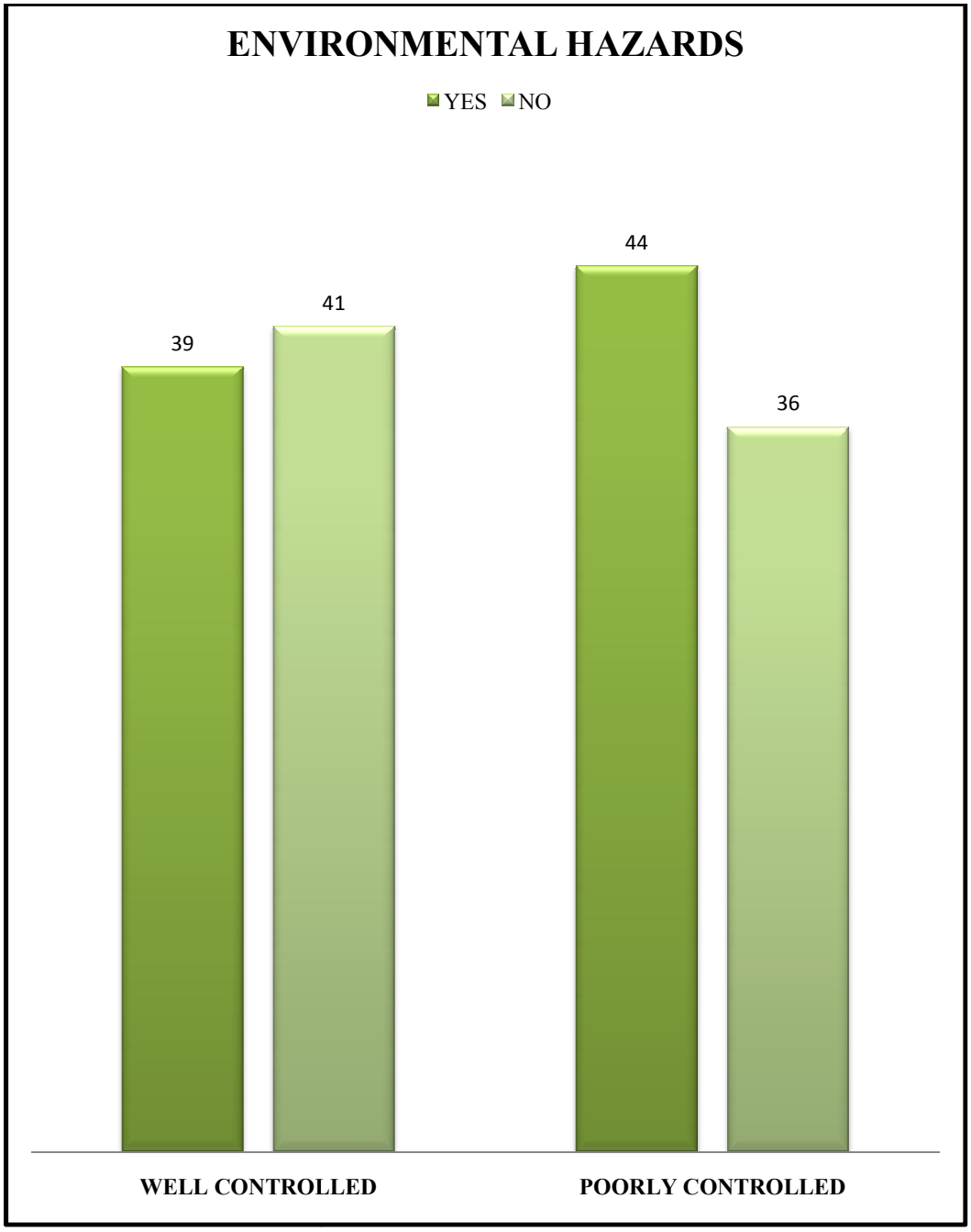


Fig 6 : Distribution of Well and Pooly controlled patients with positive family history

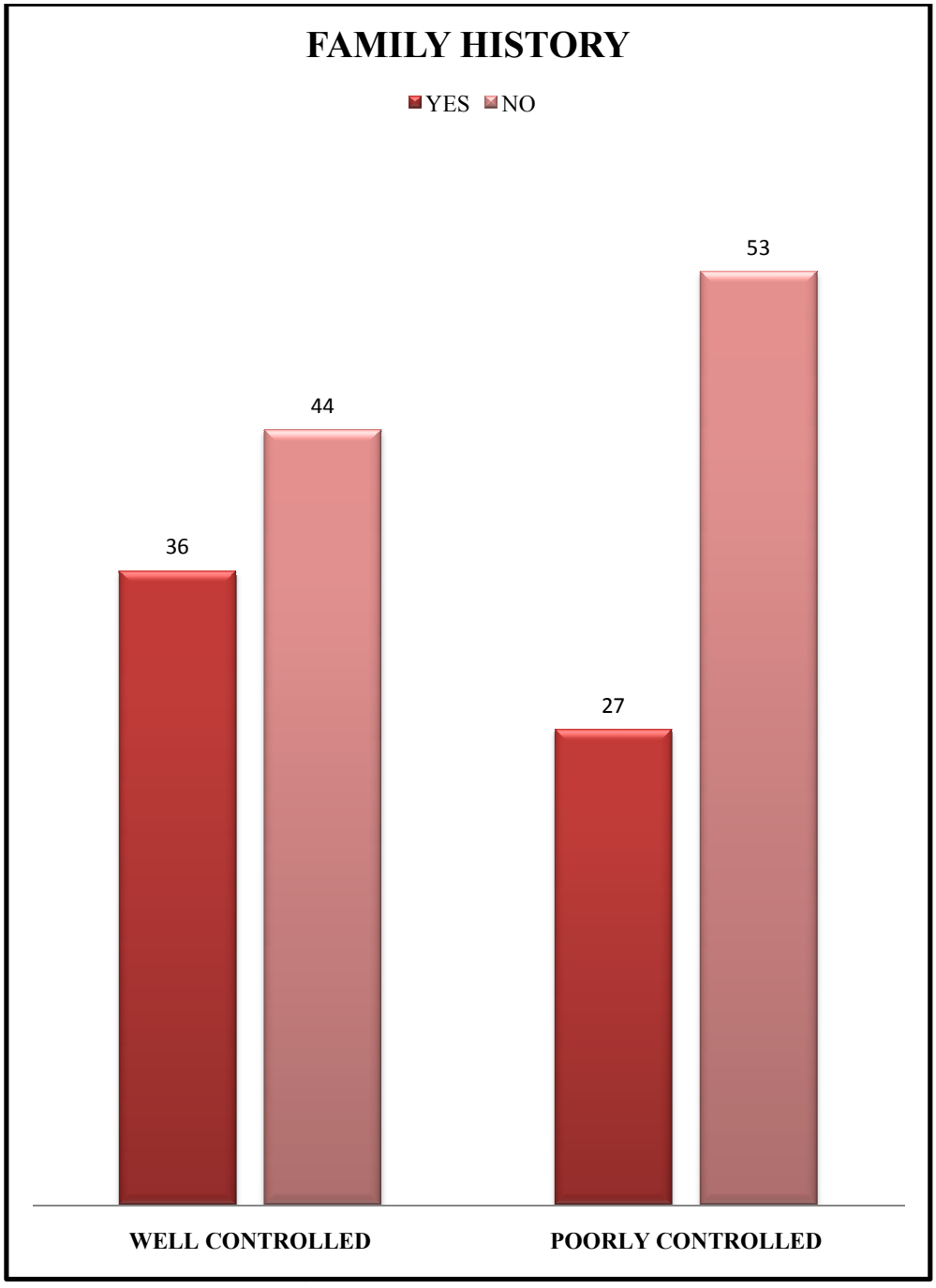


Fig 7 : Distribution of Well and poorly controlled patients who take regular treatment

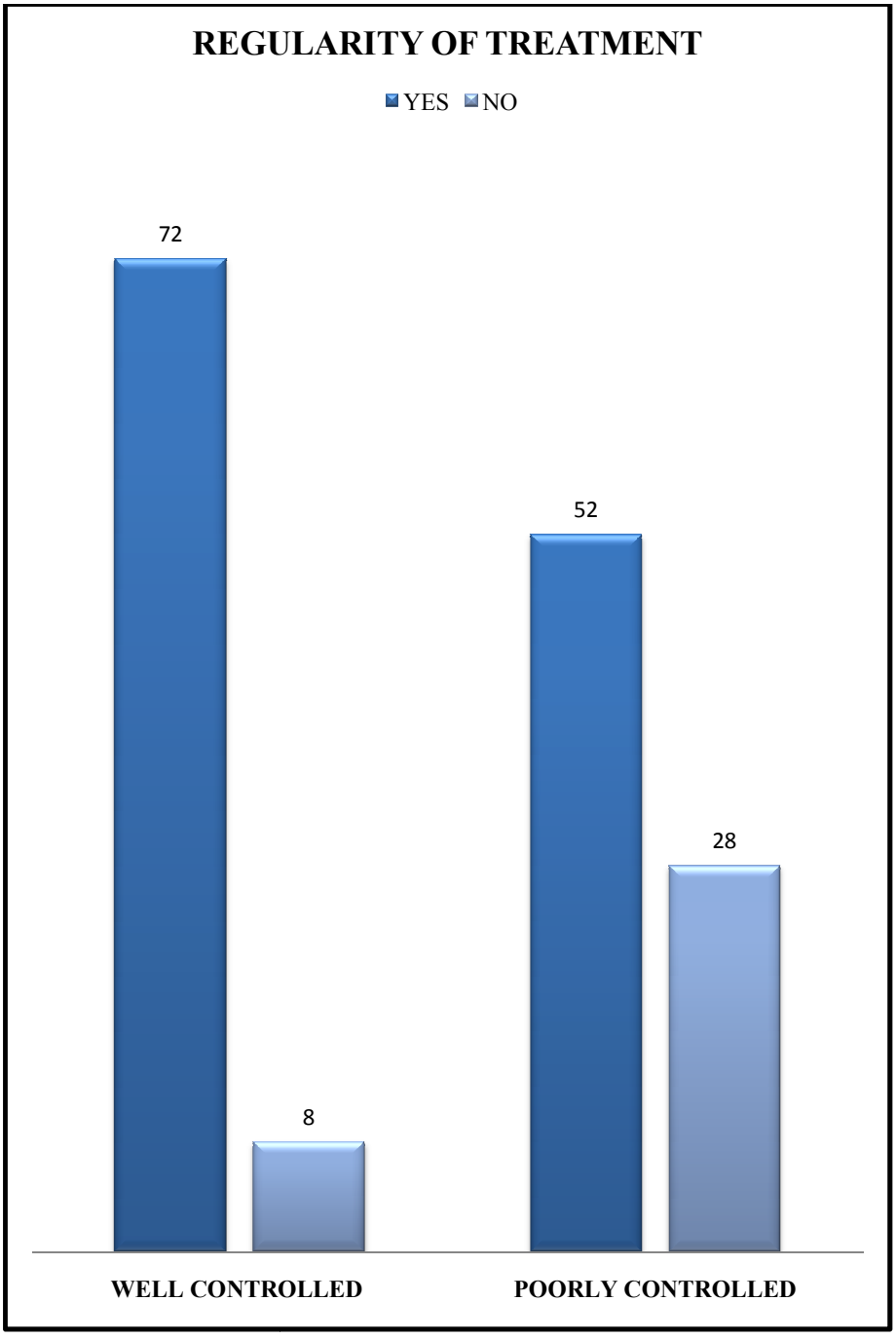


Fig 8 : Distribution of Well and poorly controlled patients with positive smoking history

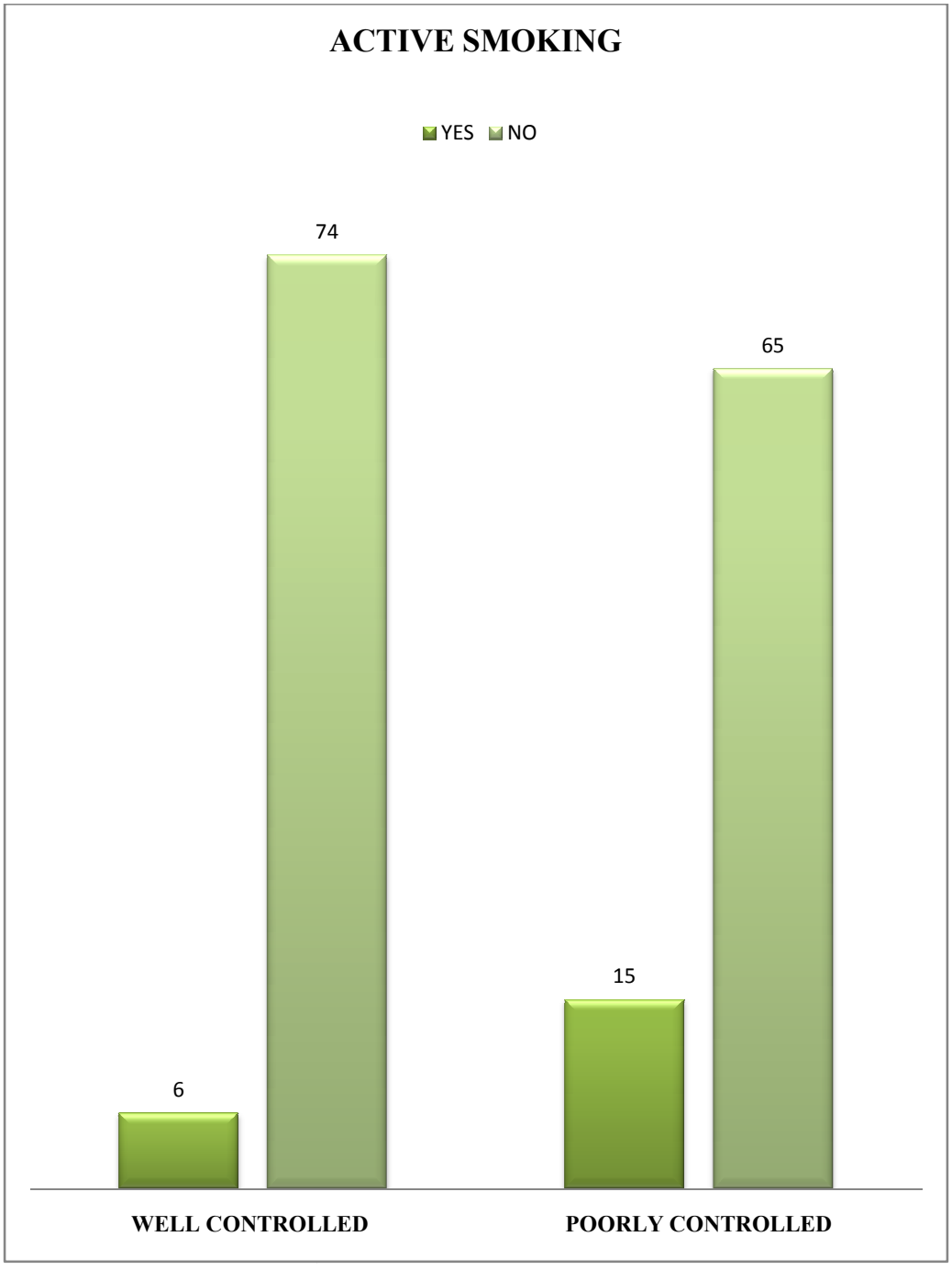


Fig 9 : Distribution of well and poorly controlled patients who are exposed to passive smoke

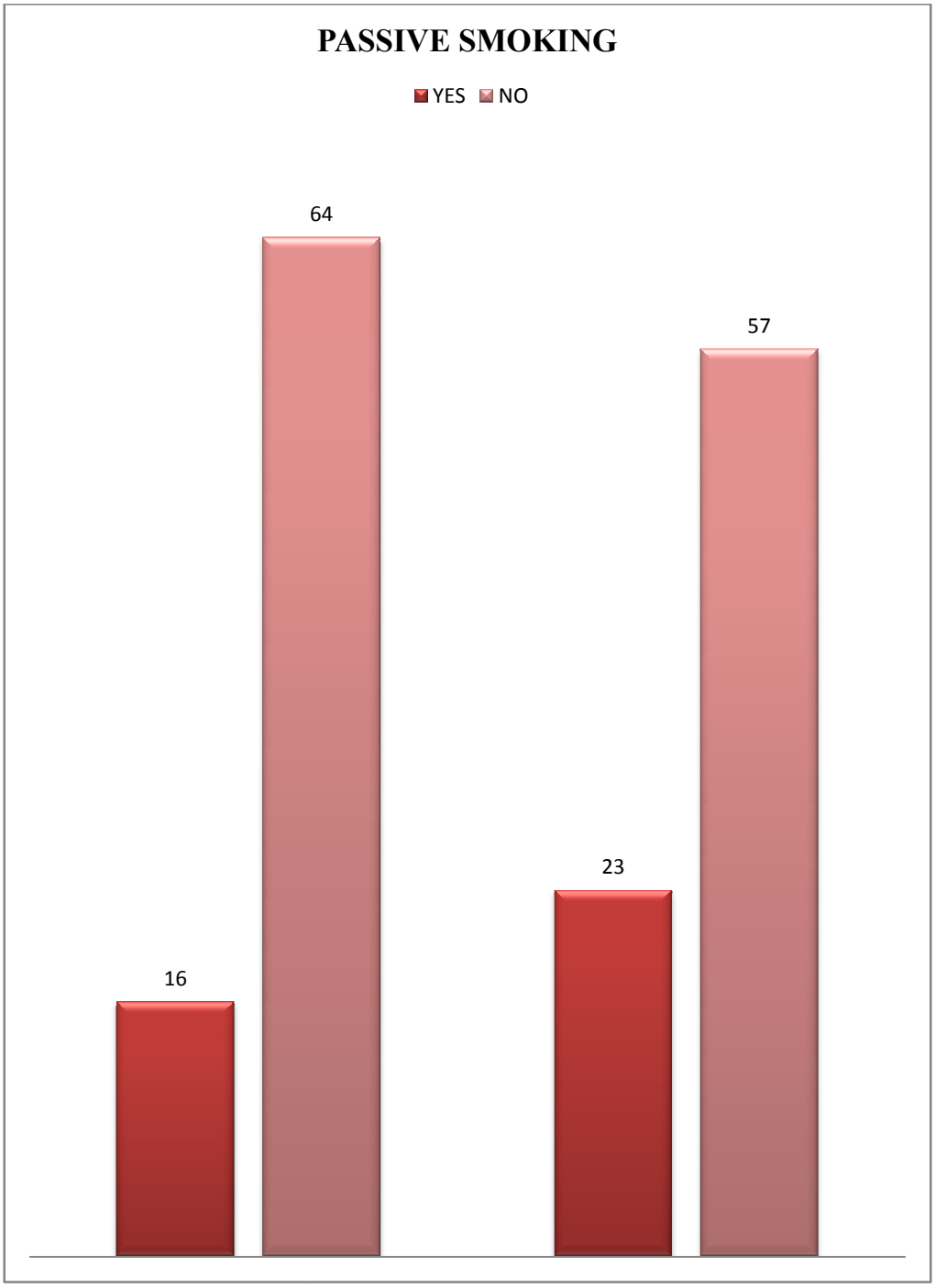


Fig 10 : Distribution of well and pooly controlled patients who are allegic to dust

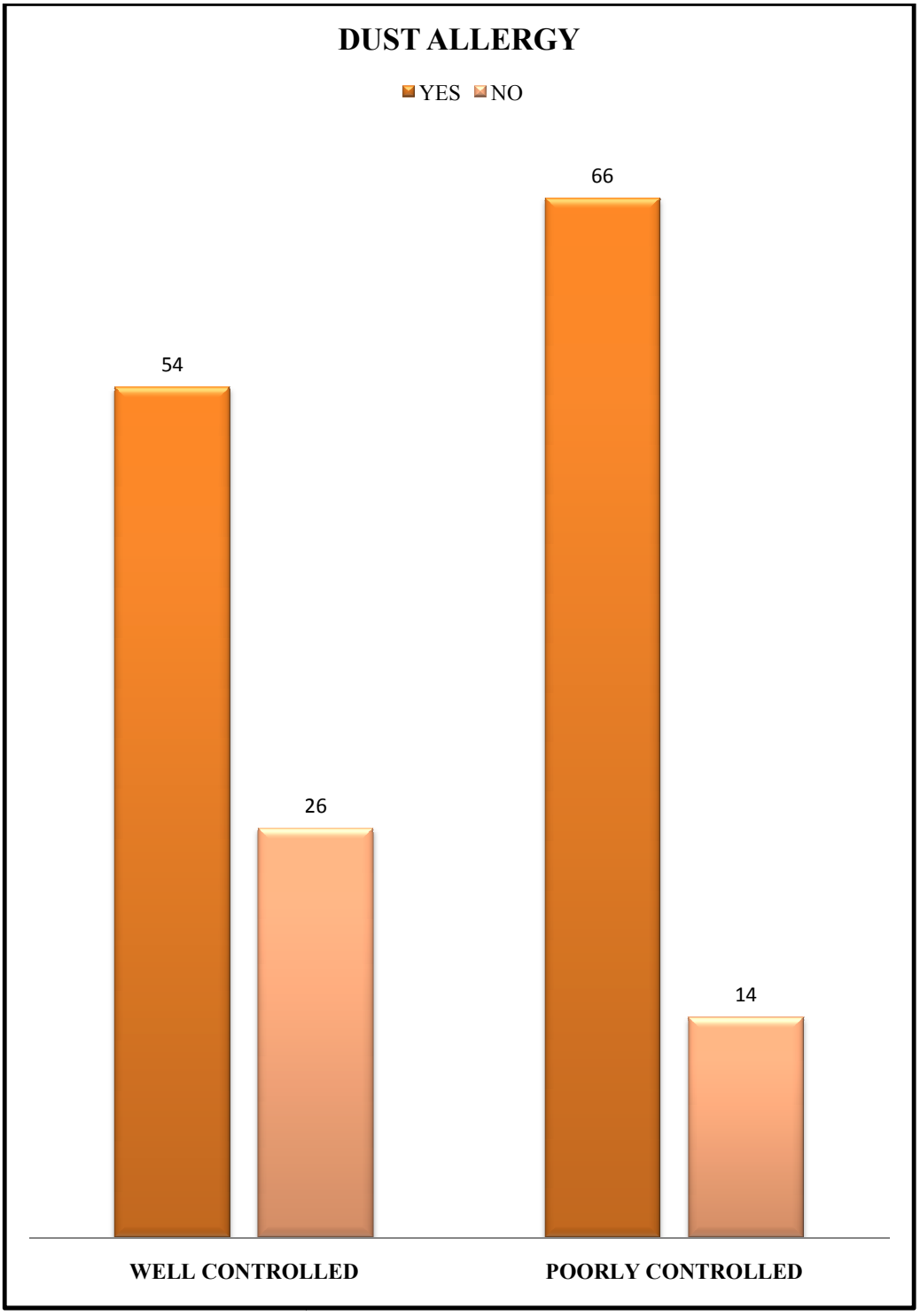


Fig 11 : Distribution of well and poorly controlled patients who are allergic to smoke

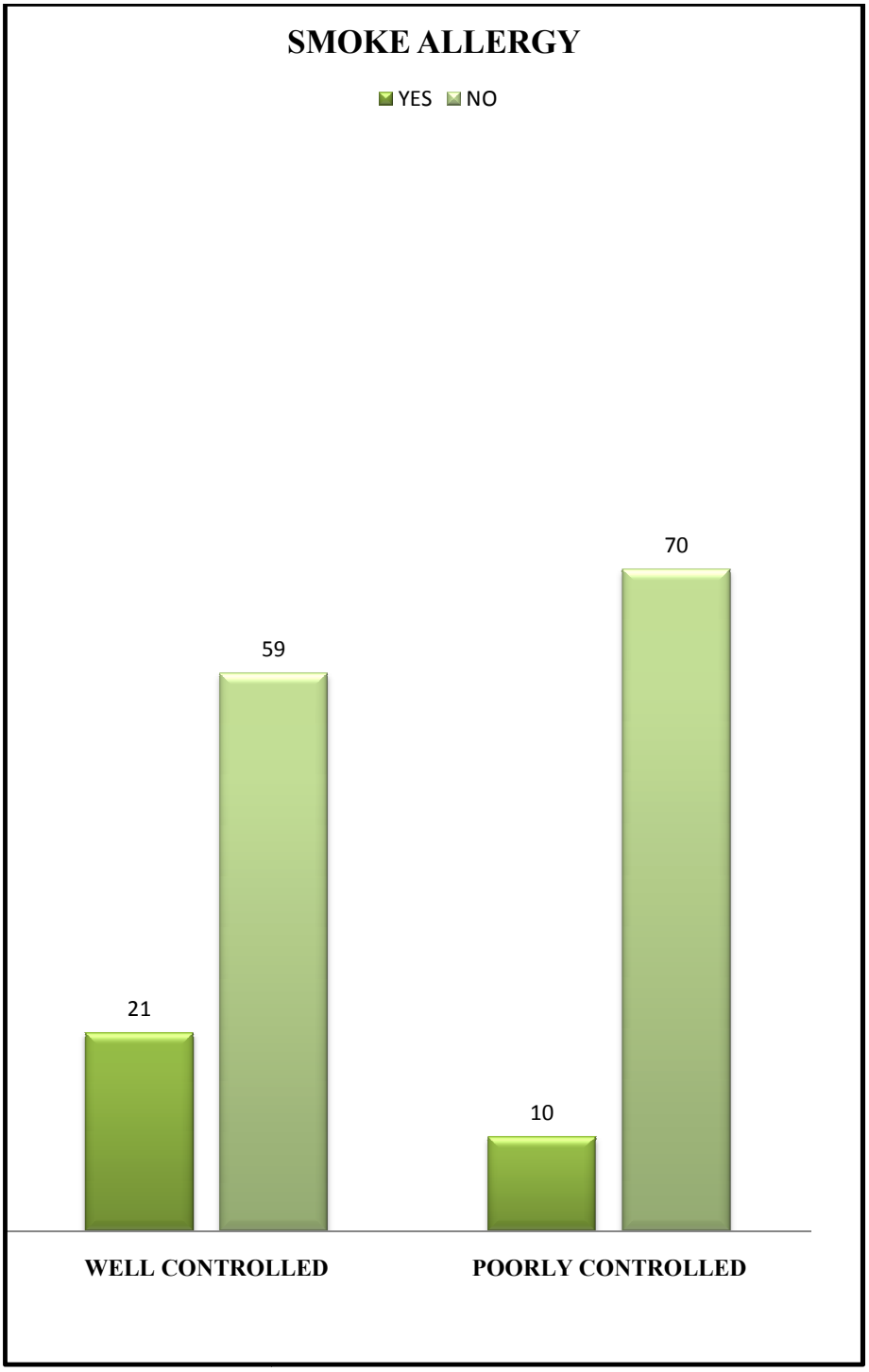


Fig 12 : Distribution of well and poorly controlled patients who have H/O Food Allergy

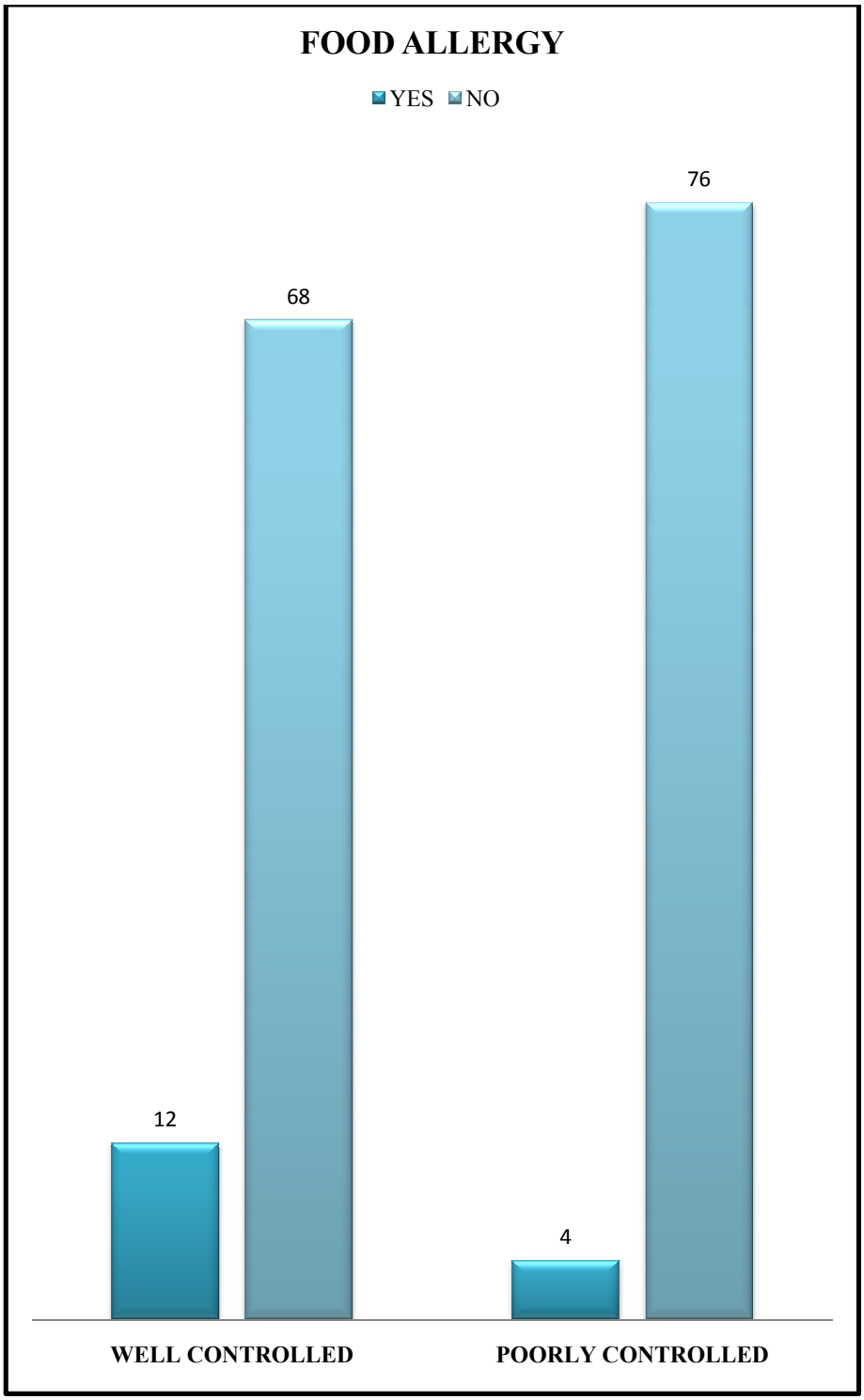


Fig 13 : Distribution of well and poorly controlled patients who are allergic to harsh smell

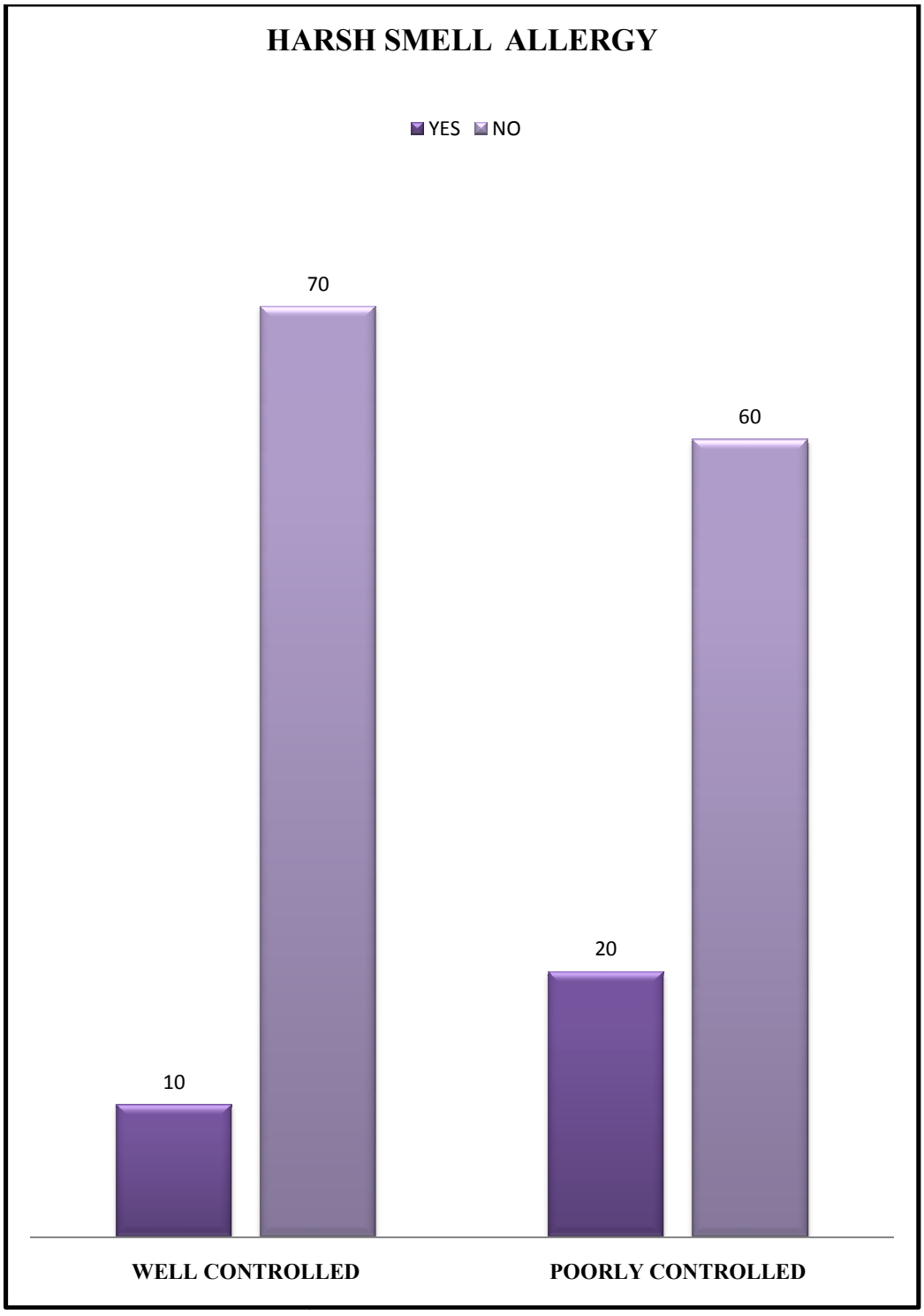


Fig 14 : Distributionn of well and poorly controlled patients who are allergic to seasonal change

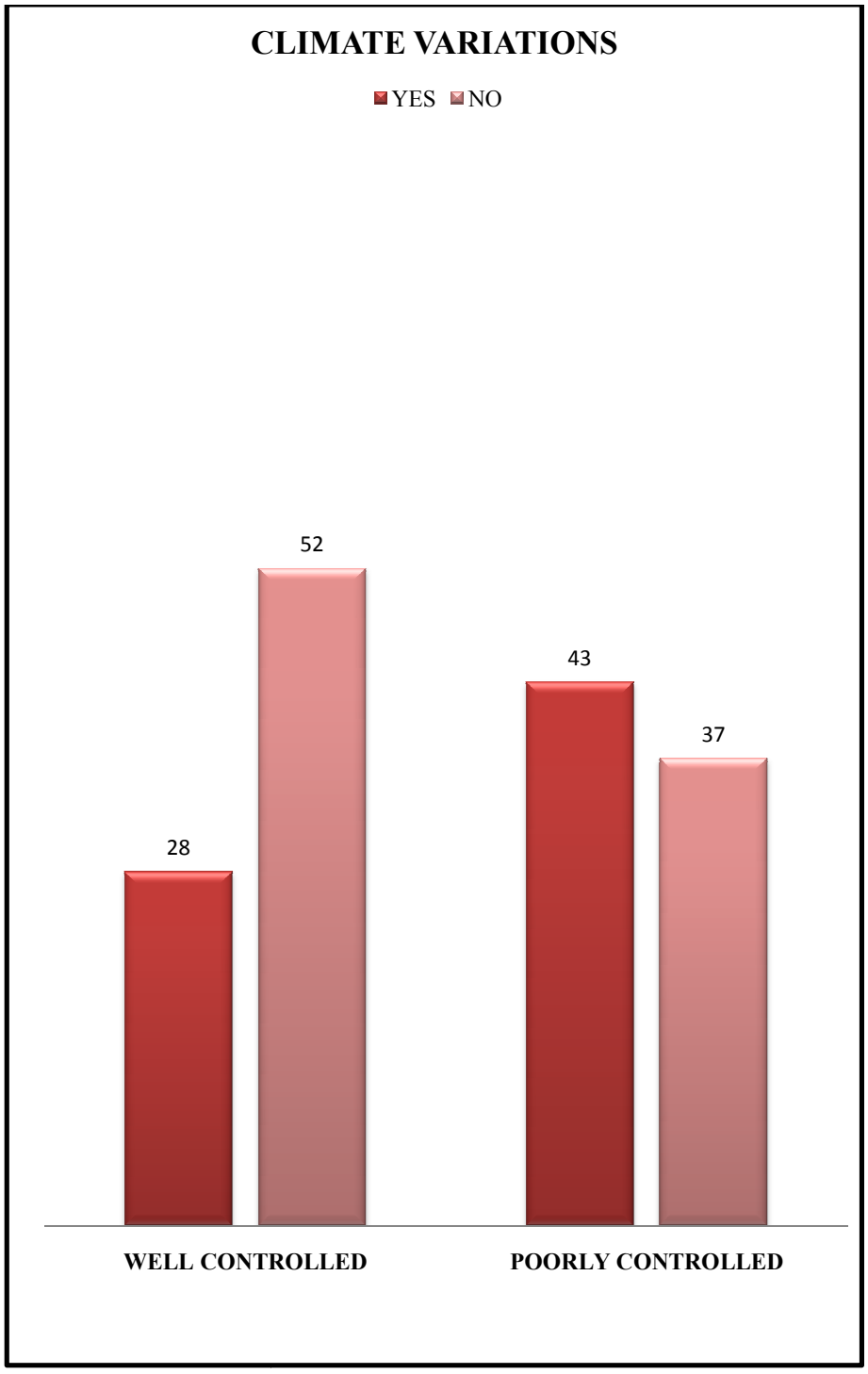


Fig 15 : Distribution of well and poorly controlled patients who have pets

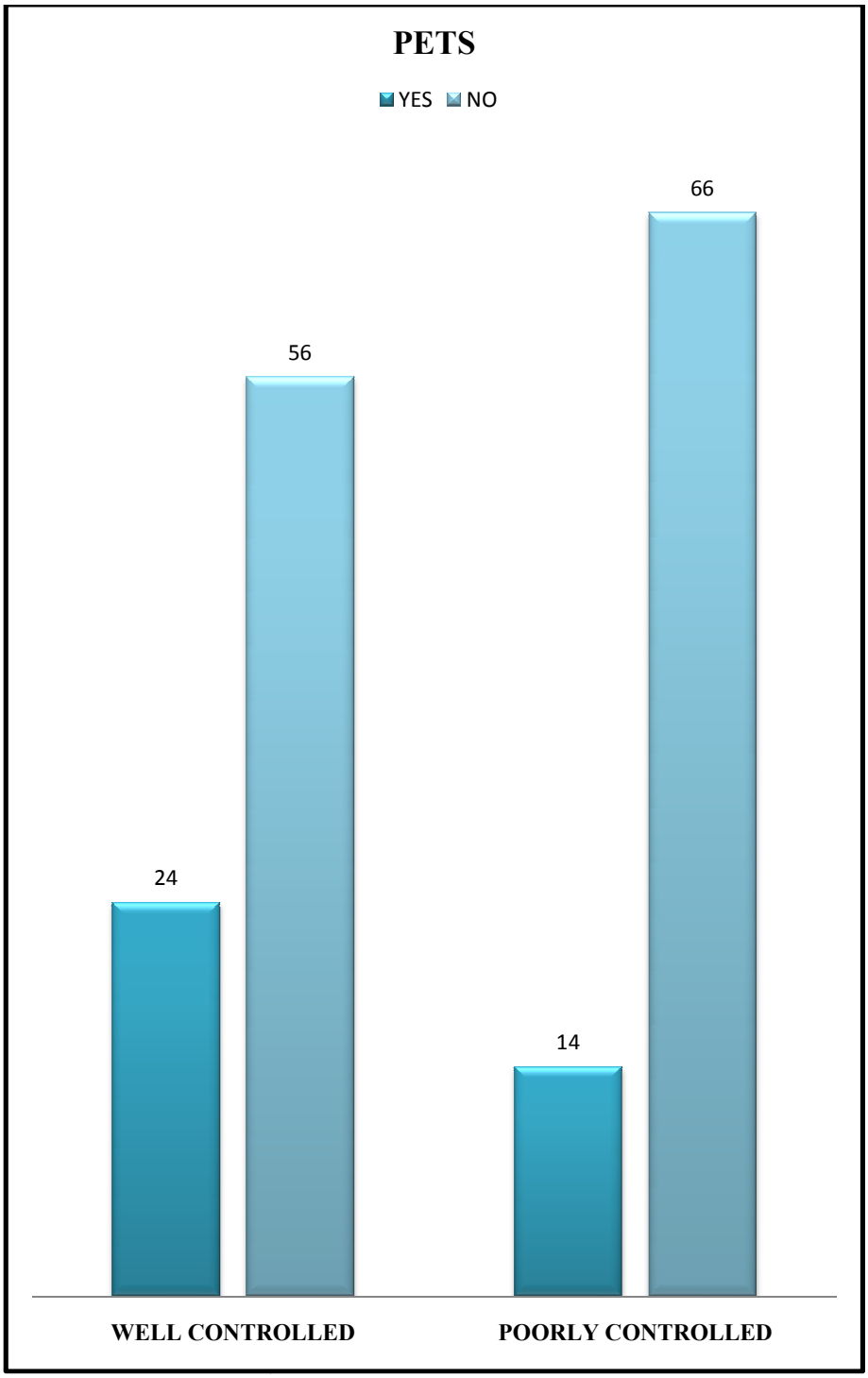


Fig 16 : Distribution of well and poorly controlled patients with symptoms of GERD

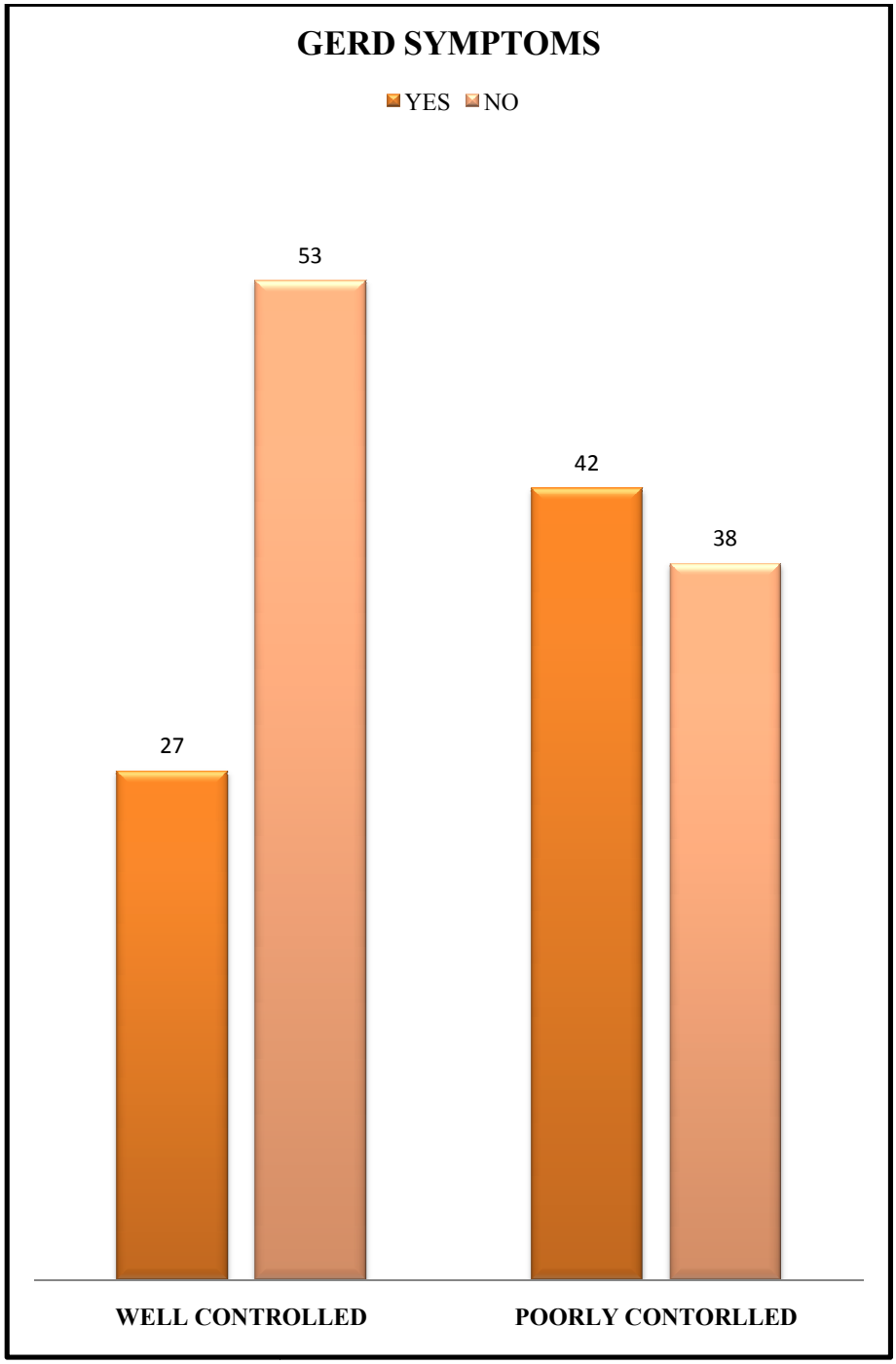


Fig 17 : Distribution of well and poorly controlled patients with symptoms of URTI

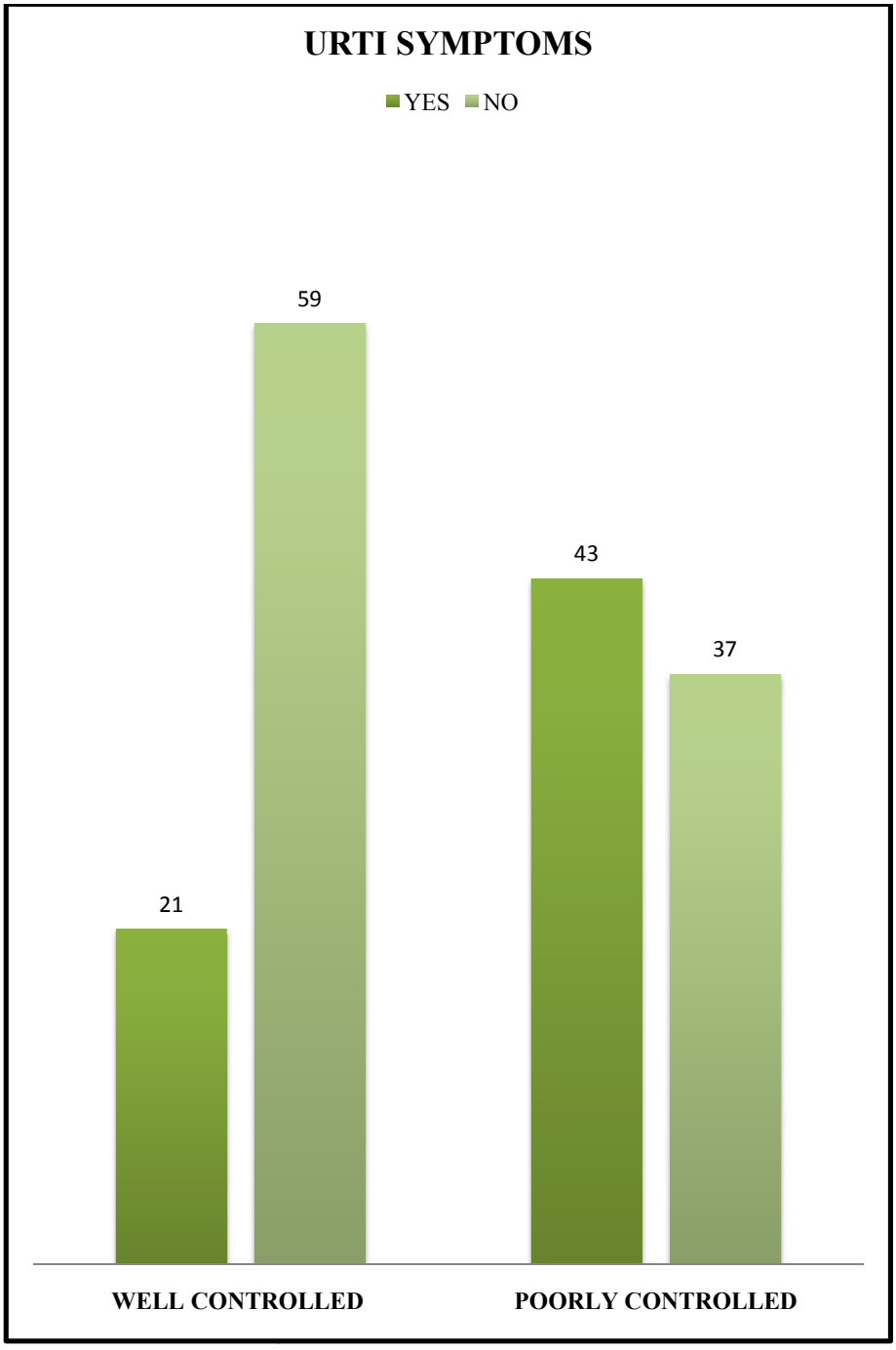


Fig 18 : Distribution of well and poorly controlled patients with symptoms of Sinusitis

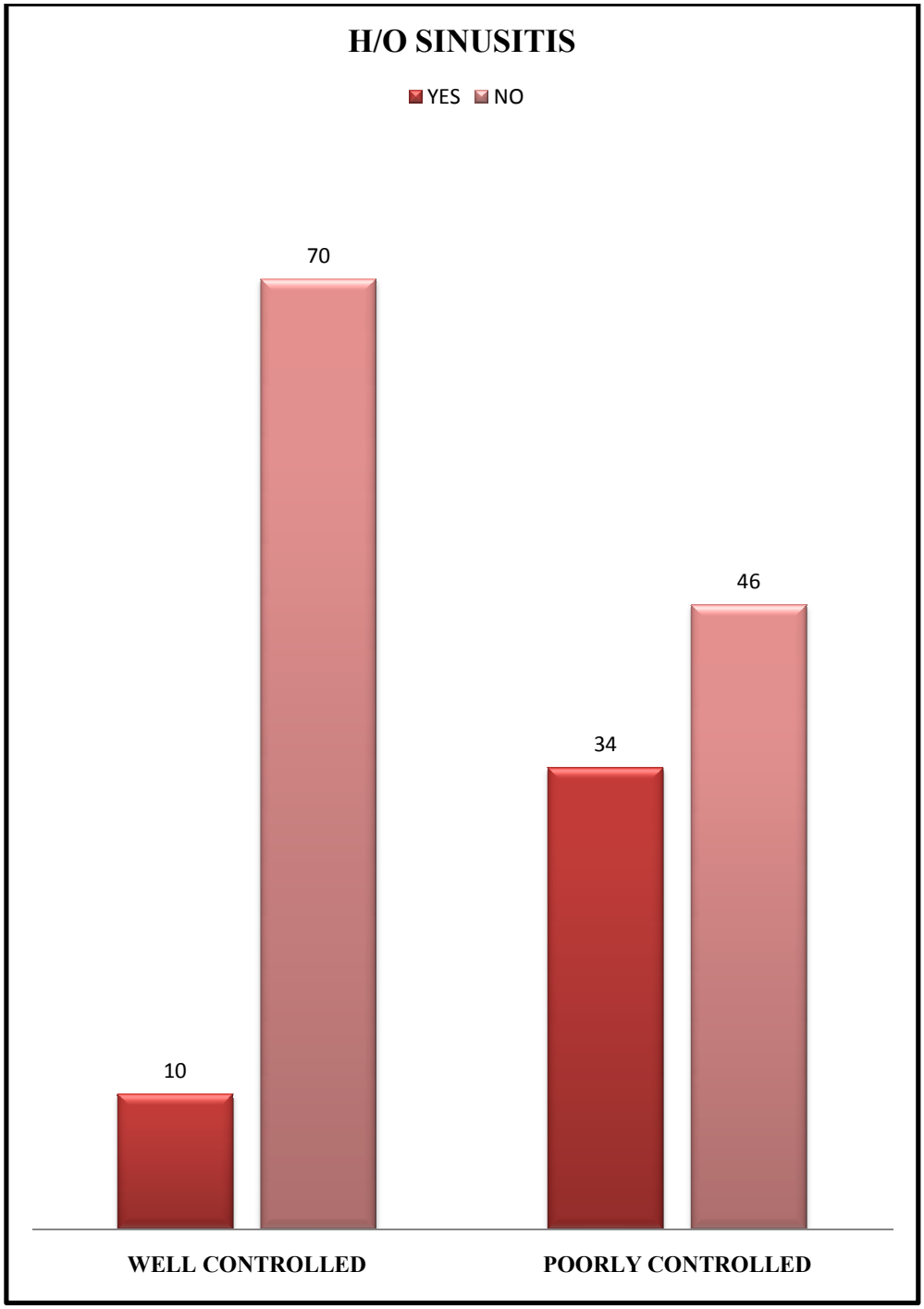


Fig 19 : Distribution of well and poorly controlled patients with positive history of admission in hospital in past one year

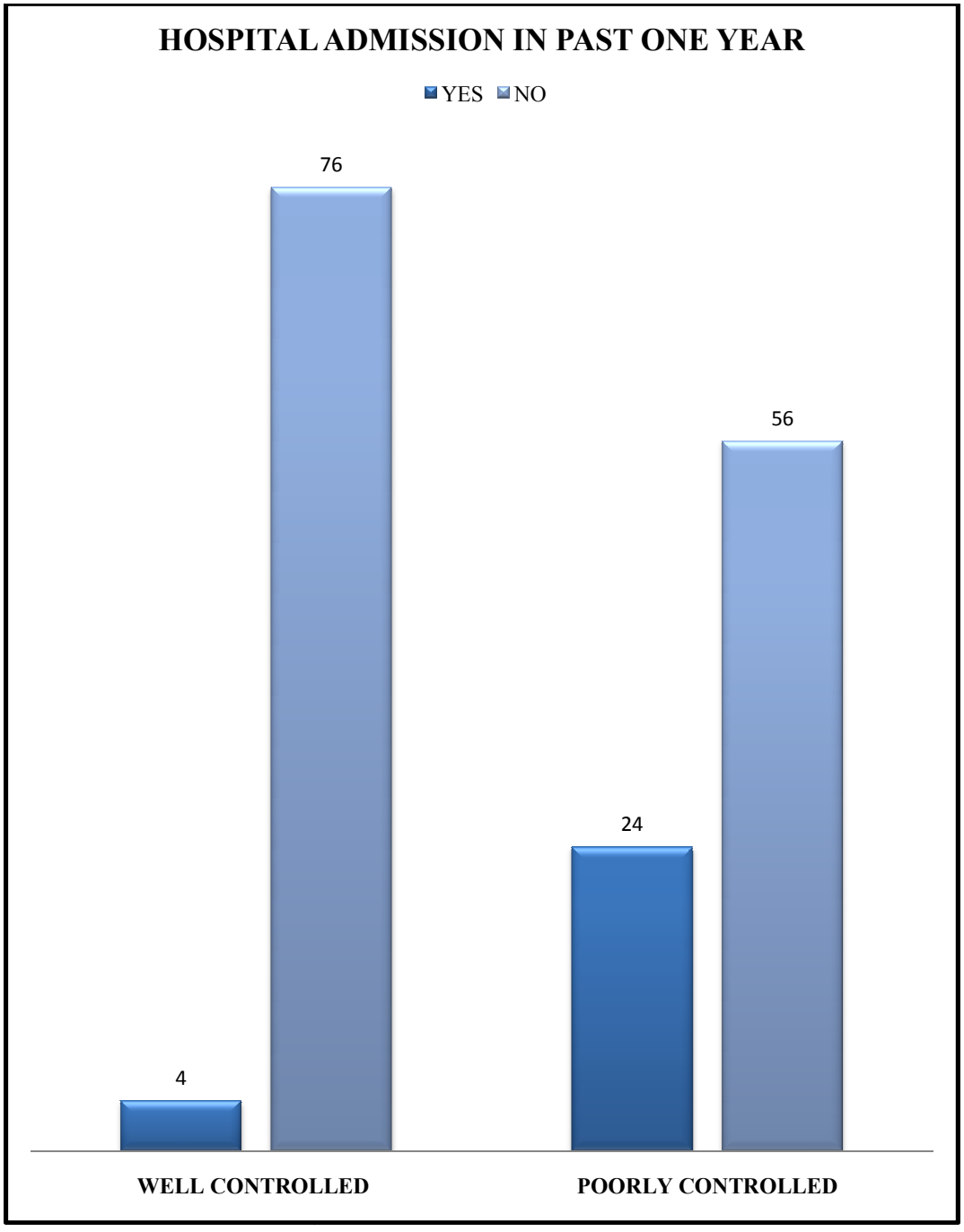


Fig 20 : Distribution of well and poorly controlled patient with history of visiting Casualty in past one year

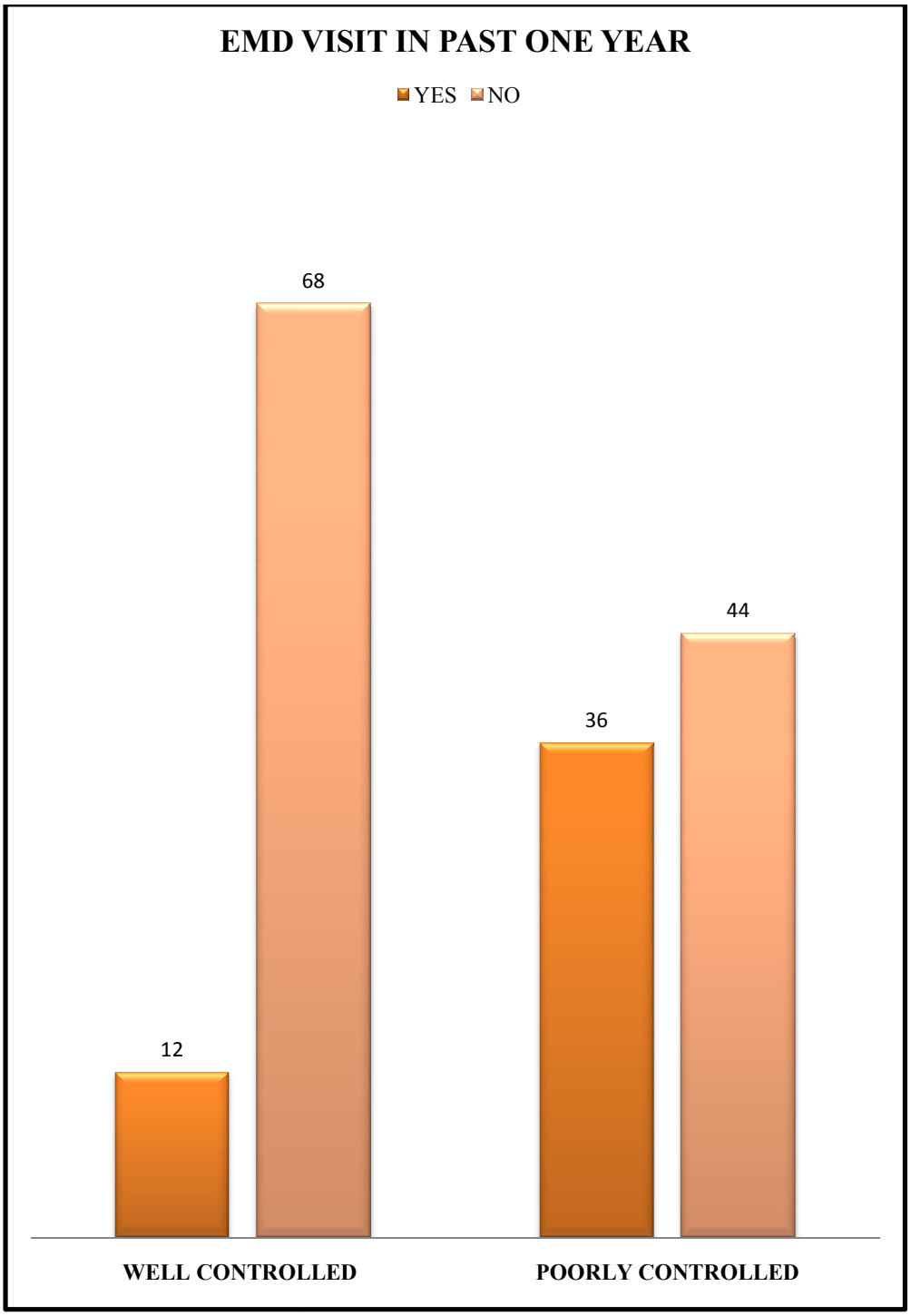


Fig 21 : Percentage of polymorphic individuals

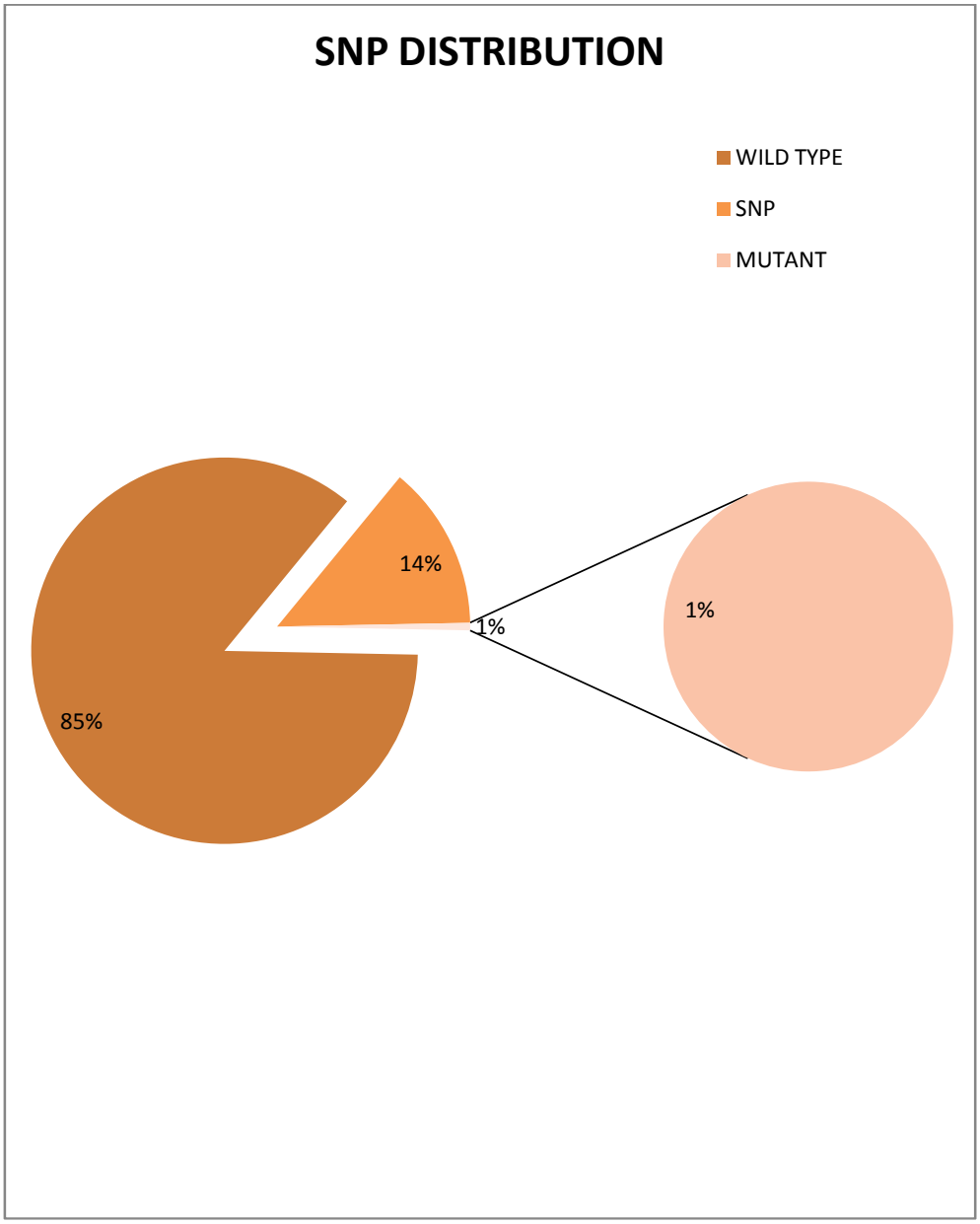
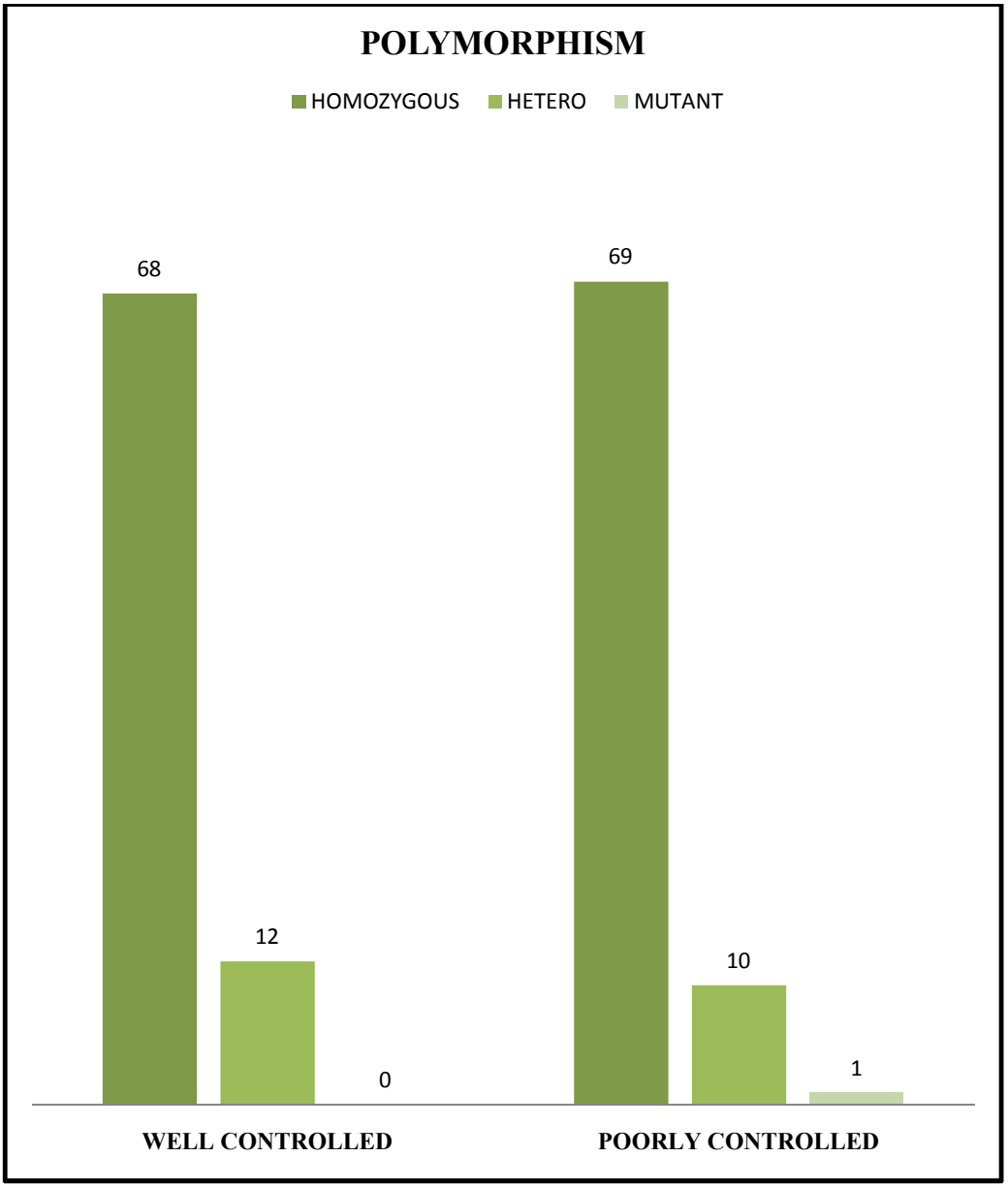


Fig 22 : Distribution of polymorphism among well controlled and poorly controlled patients



STATISTICS TABLE

Variable	P Value	OR	CI-Lower	CI-Upper
AGE	0.409	1.314	0.687	2.514
BMI	0.205	1.495	0.801	2.79
FAMILY HISOTRY	0.035	0.502	0.262	0.958
REGULAR TREATMENT	0.000	0.206	0.087	0.489
GERD	0.017	2.17	1.146	4.107
ACTIVE SMOKING	0.035	2.846	1.043	7.765
PASSIVE SMOKING	0.197	1.614	0.777	3.353
ENVIRONMENT HAZARDS	0.429	1.259	0.691	2.392
DUST ALLERGY	0.028	2.27	1.081	4.771
CLIMATE VARIATION	0.017	2.158	1.143	4.076
SMOKE ALLERGY	0.028	0.401	0.175	0.919
FOOD ALLERGY	0.035	0.298	0.092	0.969
HARSH SMELL ALLERGY	0.043	2.333	1.014	5.371
URTI	0.000	3.265	1.680	6.344
SINUSITIS	0.000	5.174	2.331	11.482
HOSPITAL ADMISSION	0.000	8.143	2.675	24.791
CASUALTY VISTS	0.000	4.636	2.178	9.868
PETS	0.063	0.495	0.234	1.047
SNP	0.822	1.107	0.456	2.68

DISCUSSION

This cross sectional study was undertaken to evaluate the association of GRK 5 single nucleotide polymorphism (GRK5 SNP) and poorly controlled asthma.

Our central hypothesis was that there would be a positive association between the GRK5 SNP and poor control of asthma, because of a reported endogenous beta blockade by this SNP[108]. We chose to study asthma because of its increasing prevalence every year and even with appropriate therapy, more number of people continues to have poor control of asthma[109].

The total number of patients recruited in the study was 160, and there were 37% males and 63% females .Among them, 53 % of males and 52 % females were poorly controlled. Previous community based studies have shown female sex as a risk factor for developing asthma[110].This was also observed in our study as the male: female ratio was 1: 1.7. In spite of this female preponderance in poorly controlled asthma group, it was not statistically significant. This has been previously noted in a different study by Dursun et al[111].

In our study we found that age did not seem to influence control of asthma as well. Though Odds Ratio from our results was 1.314 indicating

that older the age greater was the chance for developing poorly controlled asthma, the confidence interval for this OR stands at 0.69 to 2.5. Therefore we could not treat this result as significant. Noteworthy was that 65 % of patients were of age group >40 years.

Though in our study population, a statistically significant difference between control of asthma in obese and non–obese subjects was not detected (p=0.205) and OR is 1.495 (95 % CI 0.801- 2.790), there is said to be an increased association of poor control of asthma with increasing BMI in previous studies. Mosen et al. concluded obesity is associated with worse asthma outcomes and also increased asthma related hospitalizations[112].The possible mechanism for this association was formulated by Shore in 2008, who demonstrated in a mouse model that obesity is a state of chronic low grade inflammation with adipose derived hormonal influence and sleep disordered breathing which is associated with decreased lung volume and tidal volume resulting in airway narrowing[113].

Curiously, family history of asthma appeared to be negatively correlated with poorly controlled asthma yielding a statistical significance of p=0.035, and an OR of 0.502 (95 % CI 0.264 to 0.958). Most of prior literature on family history of asthma seems to be prevalence studies rather than examining association between family history of asthma and

its control. From our study, patients with positive family history are found to have better control compared to those without a positive family history. This could have been due to increased awareness in the family about the disease, its outcome and treatment modalities and the need for therapeutic compliance. Thus, the patients in our study with a positive family history may have achieved a good control of asthma.

Our data for compliance of treatment and poor control shows a sturdy association ($p < 0.001$) with OR 0.206 and Confidence interval falling between 0.087 and 0.489. This implies that patients with regular treatment history are well controlled. This is understandable from the fact that in asthma, compliance improves outcome. A recently published population based cohort study in Canada showed that people adherent to treatment had less exacerbation, less hospitalizations, lower health care utilizations and need for less rescue medications[114].

On the basis of medications 25 % used inhaled corticosteroids maintenance monotherapy, 55% used inhaled corticosteroid and 20% used additional anticholinergic medication at the time of study recruitment. But there was no significant difference among the patients taking only ICS (Inhaled corticosteroids) or ICS + LABA (long acting beta agonist) or ICS + LABA + Anticholinergic .This is similar to a

Canadian study in which proportion of controlled and uncontrolled patients were same irrespective of their medication used[115].

It was also found that patients who had history of hospitalizations ($p<0.001$) OR 8.143 (CI 2.7-24.7) and casualty visits ($p<0.001$) with OR 4 (CI 2.2 – 9.9) in past one year fell under the poorly controlled arm. This is an expected statistical association because these are used as diagnostic criteria for poorly controlled asthma.

Other co morbid conditions like hypertension, diabetes and tuberculosis were not found to be significantly associated with control of asthma. It is remarkable that none of the patients were treated with beta blockers for hypertension among the study participants. This could be reflected from fact that beta blockers are currently not the first line of drug for hypertension and moreover use of beta blockers are essentially avoided by clinician treating patients with asthma. It was also noted that common anti hypertensive drugs used were amlodipine and telmisartan, and anti diabetic drugs used were glimepride and metformin.

In our study population about 14% were smokers and 24% had history of passive smoking. Passive smoking recipients included co workers and those who had husbands who smoke. The features associated with smokers and asthma are increased symptoms and disability coupled with accelerated decline in FEV_1, therapeutically reduced response to

corticosteroids and pathological alteration in airways with inflammation and increased bronchial secretion[116]. Our results revealed statistically significant association between active smoking and poor control but not significant for passive smokers.

Similarly exposure to other environmental pollutants also plays a role as risk factor for asthma. Our study participants were mostly patients residing near cotton mills, rice mills, working in textile industry and exposed to farming dust. Surprisingly the association between control and that of these hazards was not statistically significant (p=0.429) with OR of 1.25(95% CI 0.690-2.392).This could be due to the fact that patients were already on controller medications, though it s detrimental and causing progression of airway inflammation in asthma, the patient may not manifest the symptoms or acute exacerbation. Similar explanation could be also offered for patients who had pets, as they showed no statistically significant difference in their asthma control.

Known allergy or atopy to dust, smoke, seasonal variation, allergic to food and harsh smell (which included perfumes, smell during sautéing food, paint fumes, onions) were considered. Some of these like dust, season and harsh smell showed statistically significant positive association for poorly controlled asthma, whereas smoke and food allergy did not show significant association.

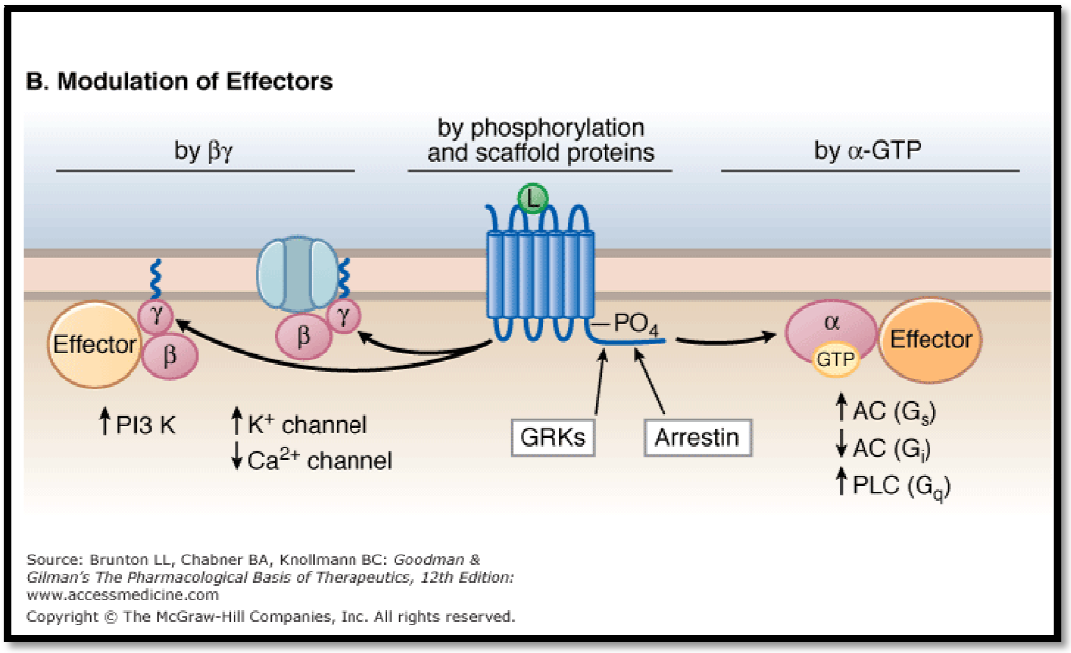
Patients with coexisting GERD showed statistically significant correlation (p=0.017) OR of 2.170 (95% CI 1.146-4.107) for poorly controlled asthma. Multiple studies have stated this positive association between GERD and difficult to treat asthma. Nearly one third asthma patients have silent reflux[117].

The rationale in support of this is due to the relaxation of the gastroesophageal sphincter as a result of bronchodilator therapy, flattening of diaphragm due to air trapping, activation of vagal reflex by cholinergic pathway and accompanied with microaspiration of stomach contents.

Also history of upper respiratory tract infection and history of sinusitis were found to have statistically significant ($p < 0.001$) relationship to poorly controlled asthma. This could be due to the grounds that these may not be causally related to control in asthma rather the result of poor control.

Beside all these factors, our central hypothesis was to examine if there would be a positive association between GRK 5 SNP and poor control, because of a demonstrated endogenous beta blockade by the SNP. Hence we suspected that if this polymorphism were present in an asthmatic individual, he/she may have a decreased activity of the drug due to a pre-exisiting beta blockade.

Picture 7 : Demonstrating the role of GRK's



Role of GRK 5

This became our gene of interest in view of its central regulatory role in physiology of bronchial smooth muscle and also because it has not been studied so far. GRK 5 gene controls the expression of the kinase and thereby regulates the GPCR. Our hypothesis that this kinase would influence asthma control was because, by literature, GRK's are necessary for down regulation of the receptors.

A polymorphism in the GRK5 isoform was discovered at codon 41 in which a wild type glutamine (Gln) 41 is replaced by leucine(Leu) .In transfected cells GRK5-Leu 41 evoked a greater degree of agonist promoted desensitization of adenyl cyclase compared to GRK5-Gln41.

Consistent with this functional effect , agonist promoted beta2-AR phosphorylation was greater in cells expressing GRK5-Leu41, similar to the rate of agonist promoted receptor internalization. So GRK5-Leu 41 represents a gain of function polymorphism that evokes enhanced loss of function of Beta2-AR during persistent agonist exposure, and thus contributing to beta agonist variability in asthma treatment.

This GRK5-Leu 41 variant is approximately 10 fold more common in African- Americans[118]. It was found that the prevalence of this polymorphism is variable between different populations depending on the ethnicity. In some races the SNP was seen in around 2 % of the population while in the African Americans it was as high as 40 % [108]. Our study population had 14 % of GRK5-Leu41 variant.

A prior study has demonstrated a higher prevalence of this SNP among African Americans compared to Caucasian[108]. Another study showed that African Americans exhibit a poorer control of asthma as well as potentially greater adverse effects during chronic treatment when compared with Caucasians[118]. Though a prospective study has not yet been conducted to demonstrate a link between the SNP and poorer control of asthma in this population, the authors of the SMART study suggest that among other things, a genetic factor may well be the underlying reason for the same.

So we were interested in looking at how GRK5 polymorphism affected asthma control among the Indian population. However, the frequency of this variant was equal among those with well controlled and poorly controlled asthma. Thus the difference in frequency distribution of this polymorphism did not show any statistical significance (p=0.822).

The reason for such an inference could be due to the small sample size of our study .Other plausible explanation could be because of the influence of the same SNP on muscarinic receptors resulting in bronchodilatation that could be exhibiting a possible functional antagonism on the effects of this SNP on beta 2 adrenoreceptors.

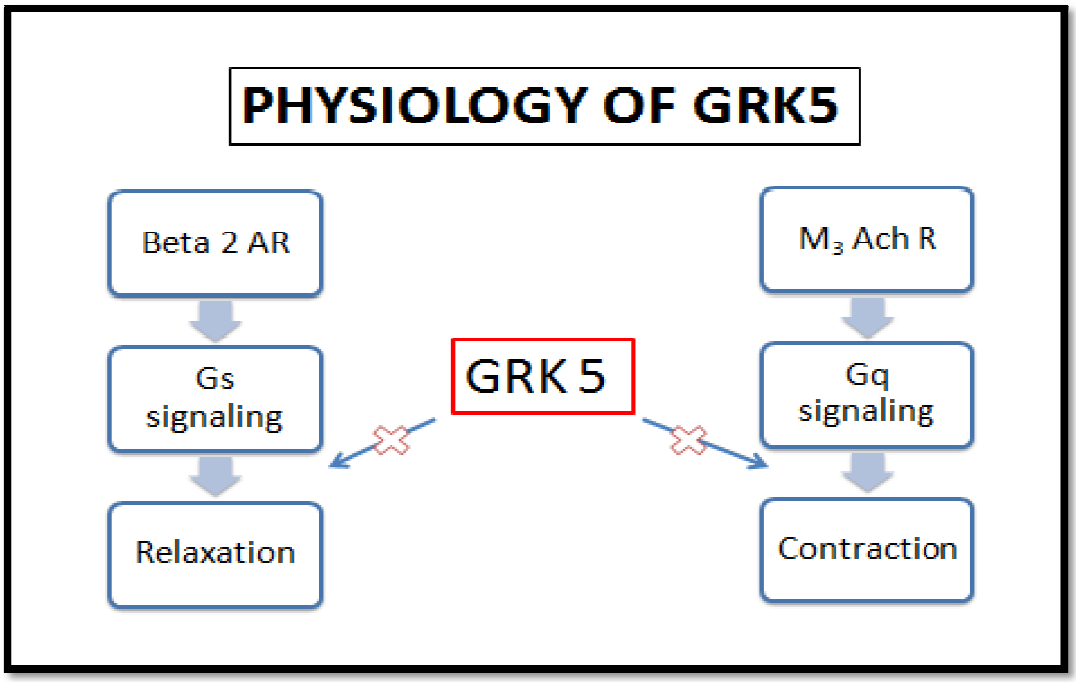
Desensitization of Beta 2 AR's through GRKs.

Beta 2 Adrenergic receptor is a GPCR, which on activation by a agonist leads to $G\alpha_S$ activation which in turn activates adenylcyclase(AC) ,which hydrolyses adenosine triphosphate(ATP) into cyclic adenosine monophosphate (cAMP) . The binding of cAMP to regulatory subunits proteins of cAMP (PKA), releases the catalytic subunit of the PKA, and activated PKA exerts numerous effects in airway smooth muscle by phosphorylating many intracellular targets.

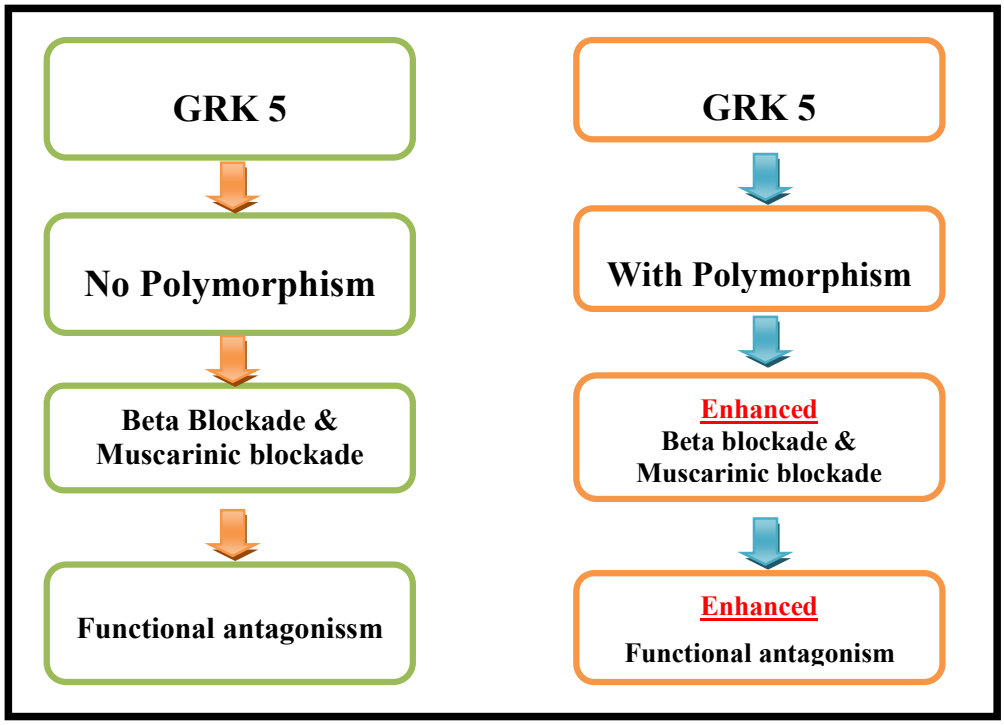
In addition to the PKA mediated β2AR signaling it is appreciated that regulatory protein arrestins can promote β2AR signaling events

distinct from those of PKA and independent of β2AR –G_S coupling. Phosphorylation of GPCR receptors by specific GPCR kinases (GRKs) plays a key role in triggering rapid desensitization. Phosphorylation of agonist-occupied GPCRs by GRKs facilitates the binding of cytosolic proteins *arrestins* to the receptor, resulting in the uncoupling of G protein from the receptor. The β-arrestins recruit proteins that limit cyclic AMP signaling, and clathrin and β2-adaptin, which promote sequestration of receptor from the membrane (*internalization*), thereby, providing a scaffold that prevens additional signaling steps. In the presence of polymorphism this desensitization and internalization is augmented.

Picture 8 : Physiological role of GRK5



Picture 9 : Role of polymorphism on GRK5



Additionally one study has demonstrated that M_2 muscarinic receptor mediated opposition of airway smooth muscle relaxation is regulated by GRK5 which is important in beta 2 receptor desensitization. In addition that study concluded GRK 5 regulates pulmonary response in a tissue specific and receptor specific manner [119].

It has further been noted that bronchial relaxation via beta 2 adrenoceptors has both direct and indirect components. The indirect component is through phosphorylation of M_3 receptors resulting in reduced production of IP_3 leading to bronchial relaxation.

It is equally possible that the functional antagonism (as discussed above) exhibited between M_3 and β_2 adrenoceptors could well underlie the fact that those with SNP were also seen in the well controlled arm.

Thus there could be variability in responses to these patients with GRK5 polymorphism either because enhanced beta blockade causing poor control or enhanced M3 mediated relaxation making them symptom free and well controlled.

Chronic use of beta adrenergic agonists is known to lead to desensitization of the receptor[88]. This desensitization could by itself result in poor control of asthma. Therefore, duration of treatment is a potential confounding factor, and this needs to be taken into account while interpreting the genetic effects.

CONCLUSION

1. This is the first study in Indian population done to assess the genotype phenotype correlation of GRK5 gene polymorphism and poor control of asthma

2. The prevalence of this polymorphism was found to be 14% in our population.

3. The current study did not demonstrate a significant association between GRK5-Leu41 polymorphism and poor control of asthma.

4. Risk factors like known allergic history to dust, smoking history, climatic variation, history of GERD, regularity of treatment were all found to have statistical significance for poor control of asthma.

5. In our study risk factors like progressing age, female sex, environmental hazard exposure, and exposure to pets were not statistically significant for poor controlled asthma.

1. Singh AB, Kumar P. Aeroallergens in clinical practice of allergy in India. An overview. *Ann Agric Environ Med* 2003; *10:* 131-6.
2. Lundback B. Epidemiology of rhinitis and asthma. *Clin Exp Allergy* 1998; *2* : 3-10.
3. Yeatts K, Sly P, Shore S, Weiss S, Martinez F, Geller A, *et al.* A brief targeted review of susceptibility factors, environmental exposures, asthma incidence, and recommendations for future asthma. *Environ Health Perspect* 2006; *114* : 634-40.
4. Masoli M, Fabian D, Holt S, Beasley R. World map of prevalence of clinical asthma. In: *Global burden of asthma.* Southmpton: Medical Research Institute of New zealand and University of Southampton; 2004. p. 12-5.
5. Aggarwal AN, Chaudhry K, Chhabra SK, D'Souza GA, Gupta D, Jindal SK, *et al.* Prevalence and risk factors for bronchial asthma in Indian adults: A multicentre study. *Indian J Chest Dis Allied Sci* 2006; *48* : 13-22.
6. Sharma SK, Banga A. Prevalence and risk factors for wheezing in children from rural areas of north India. *Allergy Asthma Proc* 2007; *28* : 647-53.
7. Cookson WOCM. Asthma genetics. *Chest* 2002; *121* :7S-13S.
8. Jaiswal A. Pets can cause asthma. *Chandigarh Tribune*2008; May: 1-2. Available from: h*ttp://www.tribuneindia.com/2008/20080507/cth1.htm*.
9. www.who.int/mediacentre/fact sheets

10. Cochrane MG, Bala MV, Downs KE, Mauskopf J, Ben-Joseph RH. Inhaled corticosteroids for asthma therapy: patient compliance, devices, and inhalation technique. *Chest* 2000;117:542–50.
11. Bateman ED, Boushey HA, Bousquet J, et al. Can guideline defined asthma control be achieved? The gaining optimal asthma control study. *Am J Respir Crit Care* Med 2004;170:836–44.
12. Harding SM. Gastroesophageal reflux: a potential asthma trigger *Immunol Allergy Clin North Am* 2005;25:131–48.
13. MacDowell AL, Bacharier LB. Infectious triggers of asthma. *Immunol Allergy Clin North Am* 2005;25:45–66.
14. Erwin EA, Platts-Mills TA. Allergens. *Immunol Allergy Clin North Am* 2005;25:1–14.
15. Tatum AJ, Shapiro GG. The effects of outdoor air pollution and tobacco smoke on asthma. *Immunol Allergy Clin North Am* 2005;25:15–30.
16. Storms WW. Unmet needs in the treatment of allergic asthma: potential role of novel biologic therapies. *J Manag Care Pharm* 2003;9:534-43.
17. Mahdi Bijanzadeh, padukudru A. Mahesh &Nallur B. Ramachandra .An understanding of the genetic basis of asthma. Indian J Med Res 134, Aug 2011, pp 149-161
18. Salter HH. Asthma: Its Pathology and Treatment, 2nd edition. London: Churchill; 1868.

19. Osler W. Diseases of the respiratory system: bronchial asthma. The Principles and Practice of Medicine, 4th edition. New York: D. Appleton and Company; 1901. pp. 628-32
20. Ashok Shah,Richa Sareen. Bronchial Asthma. In : D Behera , SN Gaur , SK Katiyar. NCCP Textbook of Respiratory Medicine. New Delhi : Jaypee ; 2011.
21. Schultz WH .Physiological studies in anaphylaxis. I: The reaction of smooth muscle of the guinea-pig sensitized with horse serum. J Pharmacol Exp Ther.1909; 1:566
22. Dunbar WP.The present state of our knowledge of hay fever. J HYg. 1913 ; 13:105
23. Rackemann FM.A clinical study of one hundred fifty cases of bronchial asthma. Arch Intern Med. 1918; 22:552.
24. Ciba Guest Symposium. Terminology, definitions and classifications of chronic pulmonary emphysema and related conditions. Thorax. 1959;14:286-99.
25. Fletcher CM, Pride NB. Defenitions of emphysema, chronic bronchitis, asthma, and airflow obstruction : 25 years on from the Ciba symposium. Thorax. 1984; 39:81-5.
26. Guidelines for management of asthma in adults: I-chronic persistent asthma. Br Med J. 1990; 301:651-3.

27. EPR. Expert panel report; Guidelines for the diagnosis and management of asthma (EPR 1991). NIH publication no.91-3642. Bethesda, MD: U.S. Department of Health and Human Services; National Institute of health ;National Heart,lung and Blood Institute; National Asthma Education and Prevention Program;1991 .
28. EPR-2. Expert panel report 2; Guidelines for the diagnosis and management of asthma (EPR-2 1997). NIH publication no.97-4051. Bethesda, MD: U.S. Department of Health and Human Services; National Institute of health ;National Heart,lung and Blood Institute; National Asthma Education and Prevention Program;1997.
29. EPR-3. Expert panel report 3; Guidelines for the diagnosis and management of asthma (EPR-3 2007). NIH publication no.08-4051. Bethesda, MD: U.S. Department of Health and Human Services; National Institute of health ;National Heart,lung and Blood Institute; National Asthma Education and Prevention Program;2007.
30. URL:http//www.ginasthma.org. Global strategy for asthma management and prevention. Updated 2009 [cited 2010 October 17].
31. William Kelly , Christine A Sorkness . Asthma . In : Joseph T Dipiro , Robert L Talbert .Pharmacotheraphy, A Pathophysiologic approach . 7th edition . China : McGraw Hill ;2008
32. Barnes PJ, Chung KF, Page CP. Inflammatory mediators of asthma: an update. *Pharmacol Rev*, **1998a**, *50*:515–596

33. Barnes PJ. Cytokine networks in asthma and chronic obstructive pulmonary disease. *J Clin Invest*, **2008a**, *118*: 3546–3556

34. Cookson WO, Moffatt MF. Genetics of asthma and allergic disease. Hum Mol Genet 2000;9: 2359–2364.

35. Barnes KC. Evidence for common genetic elements in allergic disease. J Allergy Clin Immunol 2000; 106: S192–S200.

36. Chan-Yeung M, Malo J. Occupational asthma. N Engl J Med 1995;333:107–12.

37. Cullinan P, Newman Taylor AJ. Aetiology of occupational asthma. Clin Exp Allergy 1997;27(suppl 1):41–6.

38. Jaakkola MS, Jaakkola JJK. Effects of environmental tobacco smoke on respiratory health in adults. Scand J Work Environ Health 2002;28(suppl 2):54–72.

39. Greer JR, Abbey DE, Burchette RJ. Asthma related to occupational and ambient air pollutants in nonsmokers. J Occup Med 1993;35:909–15.

40. Flodin U, Jönsson P, Ziepgler J, et al. An epidemiologic study of bronchial asthma and smoking. Epidemiology 1995;6:503–5.

41. Jaakkola MS, Piipari R, Jaakkola N, et al. Environmental tobacco smoke and development of adult-onset asthma: a population-based incident case-control study. Eur Respir J 2001; 14(suppl):P1906.

42. Castranova V, Robinson V, Frazer D. Pulmonary reactions to organic dust exposures: development of an animal model. Environ Health Perspect. 1996; 104:41–53.
43. Buck M, Wall J, Schachter E. Airway constrictor response to cotton bract extracts in the absence of endotoxin. Br J Ind Med. 1986; 43:220–226.
44. Lai PS, Fresco JM, Pinilla MA, et al. Chronic endotoxin exposure produces airflow obstruction and lung dendritic cell expansion. Am J Respir Cell Mol Biol. 2012; 47:209–217.
45. Van der Zee SC,Hoek G , Bosen HM , et al . . Acute effects of urban air pollution on respiratory helath of children and without chronic respiratory symptoms . Occup Environ Med 1999; 56:802 -12
46. Buchdahl R, Parker A, Stebbings T,Babiker A.Association between air pollution and acutechildhood wheezy episodes:prospective observational study . BMJ 1996 ;312:661-5
47. Kesten S,Szalai J.Dzyngel B . Air quality and the frequency of emergency room visits for asthma. Ann Allergy Asthma Immunol 1995; 74:269-73
48. Bates DV, Sizto R.Air pollution and hospital admissions in southern Ontario: the acid summer haze effect. Environ Res 1987; 43:317-31
49. Ponka A,Viratanen M.Chronic bronchitis, emphysema, and low level air pollution in Helsinki, 1987-1989. Environ Res 1994; 65:201-17
50. Anderson HR, Limb ES, Bland JM, et al. Health effects of an air pollution episode in London, December 1991.Thorax 1995; 50:1188-93

51. Saldiva PH, Lichtenfels AJ, Paiva PS, et al. Association between air pollution and mprtality due to respiratory diseases in children in Sao Paulo, Brazil: a preliminary report,Environ Res 1994; 65:218-25
52. Braun-Fahrlander C, Ackermann-Liebrich U, Schwartz J, et al. Air pollution and repiratory symptoms in preschool children.Am Rev Respir Dis 1992; 145:42-7
53. Mukala K, Pekkanen J, Tiittanen p,et al. Seasonalexposure to NO2 and repiratory symptoms in preschool children. J Exposure Anal Environ Epidemiol 1996; 6:197-210
54. Frischer T, Studnicka M, beer E , Neumann M. The effects of ambient NO2 on lung function in primary school children. Environ Res 1993;62 :179-88
55. Scarlett Jf, Abott KJ, Peacock JL, et al. Acute effects of summer air pollution on respiratory function in primary school children in southern England. Thorax 1996; 51:1109-14
56. Advisory group on the Med aspects of air pollution episodes.Oxides of nitrogen (3rd report). London:Department of Health, 1993:29-49
57. Hill DJ, Firer MA, Shelton MJ, Hosking CS. Manifestations of milk allergy in asthmatic children. J Paediatr 1986; 109:270-6
58. Dawson KP, Ford RPK, Mogridge N. Childhood asthma: what do parents add or avoid in children's diet? NZ Med J 1990; 103:239-40
59. Woods RK, Abramson M, Raven JM, et al. Reported food intolerance and respiratory symptoms in young adults. Eur Respir J 1998; 11:151-5

60. Bock SA, Aitkens FM. Patterns of food hypersensitivity during sixteen years of double blind placebo controlled food challenges. J Paediatr 1990; 117:561-7

61. Harding SM, Guzzo MR, Richter JE. 24 hr esophageal pH testing in asthmatics: respiratory symptom correlation with esophageal acid events. Chest 1999; 115:654-9

62. Hardings SM, Richter JE, Guzzo MR, et al.Asthma and gastroesophagela reflux: acid suppressive therapy improves asthma outcome. Am J Med 1996; 100:395-405

63. Kiljander TO,Salomaa ER, Hietanen EK, Terho EO. Gastroesophageal reflux in asthmatics: a double blind, placebo controlled crossover study with omeprazole. Chest 1999; 116:1257-64

64. Coughlan JL, Gibson PG, Henry RL. Medical treatment for reflux oesophagitis does not consistently improve asthma control: a systematic review. Thorax 2001; 56:198-204

65. Oliveira, MJoR; Pereira, AnS; es, LG., et al. Chronic exposure of rats to cotton-mill-room noisechanges the cell composition of the tracheal epithelium. Journal of Occupational and Environmental Medicine. 2002; 44:1135–1142.

66. Martinez FD, Antognoni G, Macri F, Bonci E, Midulla F, De Castro G, Ronchetti R. Parental smoking enhances bronchial responsiveness in nine-year-old children. *Am Rev Respir Dis* 1988;138:518–523.

67. Kauffman F, Annesi I. Tobacco smoke in bronchial responsiveness. In: Ilirsch A, editor. Prevention of respiratory disease. New York: Marcel Dekker; p. 1–24.

68. O'Connor GT, Sparrow D, Segal MR, Weiss ST. Smoking, atopy, and methacholine airway responsiveness among middle aged and elderly men: the Normative Aging Study. *Am Rev Respir Dis* 1989;140:1520–1526.

69. Kiviloog J, Irnell L, Eklund G. The prevalence of bronchial asthma and chronic bronchitis in smokers and non-smokers in a representative local Swedish population. *Scand J Respir Dis* 1974;55:262–276.

70. Dodge RR, Burrows B. The prevalence and incidence of asthma and asthma-like symptoms in a general population sample. *Am Rev Respir Dis* 1980;122:567–575.

71. Martinez FD, Cline M, Burrows B. Increased incidence of asthma in children of smoking mothers. *Pediatrics* 1992;89:21–26.

72. Evans D, Levison MJ, Feldman CH, Clark NM, Wasilewski Y, Levin B, Mellins RB. The impact of passive smoking on emergency room visits of urban children with asthma. *Am Rev Respir Dis* 1987;135:567–572.

73. Weiss ST, Tager IB, Speizer FE, Rosner B. Persistent wheeze: its relation to respiratory illness, cigarette smoking, and level of pulmonary function in a population sample of children. *Am Rev Respir Dis* 1980; 122:697–707.

74. Weiss ST. Smoking and asthma. *Compr Ther* 1994;20:606–610.

75. O'Connor GT, Weiss ST, Tager IB, Speizer FE. The effect of passive smoking on pulmonary function and nonspecific bronchial responsiveness in a population-based sample of children and young adults. *Am Rev Respir Dis* 1987;135:800–804.

76. Kennedy SM, Elwood RK, Wiggs BJ, Pare PD, Hogg JC. Increased airway mucosal permeability of smokers: relationship to airway reactivity. *Am Rev Respir Dis* 1984;129:143–148.

77. Gerrard JW, Cockcroft DW, Mink JT, Cotton DJ, Poonawala R, Dosman JA. Increased nonspecific bronchial reactivity in cigarette smokers with normal lung function. *Am Rev Respir Dis* 1980;122:577–581.

78. Jaackkola MS, Piipari R, Jaakkola N, Jaakkola JJ, Environmental tobacco smoke and adult onset asthma: a population based incident case-control study. *American Journal of Public Health* 2003 Vol. 93 pp. 2055- 2066.

79. Chalmers G. Macleod K. Little S. Thomson L. McSharry C and Thomson N. Influence of cigarette smoking on inhaled corticosteroid treatment in mild asthma. *Thorax.* 2002 Vol. 57. pp. 226-230.

80. Gillard F. Islam T. Berhane K. Gauderman W. McConnell R. Avol E. and Peters J. Regular smoking and asthma incidence in adolescents. *American Journal of Respiratory and Critical Care Medicine.* Vol 174. pp. 1094-1100.

81. Eisner M. Yellin E. Trupin L and Blanc P. Asthma and smoking status in a population-based study of California adults. *Public Health Reports Vol. 116.* pp 48-157.

82. Boulet L. Lemiere C. Archambault F. Carrier G. Descary M. Deschesnes F. Smoking and asthma: clinical and radiologic features, lung function, and airway inflammation. *Chest* 2006 Vol 129. No. 3 pp. 661-8.

83. Thomson N. Chaudhuri R and Livingston E. Asthma and cigarette smoking. *European Respiratory Journal* 2004 Vol. 24 pp. 822-833.

84. Chaudhuri R. Livingston E. McMahon A. Lafferty J. Fraser I. Spears M. McSharry C and Thomson N. Effects of smoking cessation on lung function and airway inflammation in smokers with asthma. *American Journal of Respiratory and Critical Care Medicine* 2006 Vol. 174 pp. 127-133.

85. Jaackkola MS, Piipari R, Jaakkola N, Jaakkola JJ, Environmental tobacco smoke and adult onset asthma: a population based incident case-control study. *American Journal of Public Health* 2003 Vol. 93 pp. 2055- 2066.

86. Stefano Guerra et al. The Relation of Body Mass Index to Asthma, Chronic Bronchitis, and Emphysema. CHEST 2002; 122:1256–1263.

87. Carlos a Camargo et al .Prospective study of body mass index and weight change in adult onset asthma in women. Arch Intern Medicine. 1999; 159:2582-88.

88. Donald K. Blumenthal, James C.Garrison. Pharmacodynamics:Molecular mechanisms of drug action. In: Laurence L.Brunton. The pharmacological basis of therapeutics. 12.China: McGraw-Hill;2011

89. Palczewski K, Kumasaka T, Hori T, et al. Crystal structure of rhodopsin: A G protein-coupled receptor. *Science,* **2000**, *289*:739–745

90. Gilman AG. G proteins: Transducers of receptor-generated signals. *Annu Rev Biochem,* **1987**, *56*:615–649

91. Ross EM, Wilkie TM. GTPase-activating proteins for heterotrimeric G proteins: Regulators of G protein signaling (RGS) and RGS-like proteins. *Annu Rev Biochem,* **2000**, *69*:795–827

92. Moore CA, Milano SK, Benovic JL. Regulation of receptor trafficking by GRKs and arrestins. *Annu Rev Physiol,* **2007**, *69*:451–482

93. Marks chesnutt, Thomas J Prendergast, Emmanuel T Tavan. Pulmonary diseases. In : Maxine A Papadakis, Stephen J Mcphee. Current medical diagnosis and treatment. 52. United states. McGraw-Hill. 2013

94. Lai CKW et al.Relationship between asthma control status ,asthma control testand urgent health care utilization in Asia .Respirology 2009;14:83-9

95. Horne R, Price D, Cleland J *et al*. Can asthma control be improved by understanding the patient's perspective? *BMC* [*Pulm.Med.* 2007; **7**: 8.]

96. Rabe KF, Adachi M, Lai CK *et al*. Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. *J. Allergy Clin. Immunol.* 2004; **114**: 40–7.

97. Lai CK, De Guia TS, Kim YY *et al*. Asthma control in the Asia-Pacific region: the Asthma Insights and Reality in Asia-Pacific Study. *J. Allergy Clin. Immunol.* 2003; **111**: 263–8

98. Boulet LP, Phillips R, O'Byrne P *et al*. Evaluation of asthma control by physicians and patients: comparison with current Guidelines. *Can. Respir. J.* 2002; **9**: 417–23.

99. Juniper EF, Chauhan A, Neville E *et al*. Clinicians tend to overestimate improvements in asthma control: an unexpected observation. *Prim. Care Respir. J.* 2004; **13**: 181–4.

100. Chapman KR, Boulet LP, Rea RM *et al*. Suboptimal asthma control: revalence, detection and consequences in general practice. *Eur. Respir. J.* 2008; **31**: 320–5.

101. Nathan RA, Sorkness CA, Kosinski M *et al*. Development of the asthma control test: a survey for assessing asthma control. *J.Allergy Clin. Immunol.* 2004; **113**: 59–65.

102. Schatz M, Sorkness CA, Li JT *et al*. Asthma Control Test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists. *J. Allergy Clin. Immunol.* 2006; **117**: 549–56.

103. Schatz M, Mosen DM, Kosinski M, Vollmer WM, Magid DJ, O'Connor E, Zeiger RS. Validity of the Asthma Control Test completed at home. Am J Manag Care 2007;13:661-7

104. Schatz M, Zeiger R, Drane A, Harden K, Cibildak A, Oosterman J, Kosinski M. Reliability and predictive validity of the Asthma Control Test administered by telephone calls using speech recognition technology. J Allergy Clin Immunol 2007;119: 336-43

105. Bateman ED, Hurd SS, Barnes PJ *et al*. Global strategy for asthma management and prevention: GINA executive summary. *Eur. Respir. J.* 2008; **31**: 143–78.

106. Li JT,Oppenheimer J, Bernstein L *et al*. Attaining optimal asthma control: a practice parameter. *J.Allergy Clin. Immunol.* 2005; **116**: S3–11.

107. Roche N, Morel H, Martel P *et al*. Clinical practice guidelines: medical follow-up of patients with asthma—adults and adolescents. *Respir.Med.* 2005; **99**: 793–815.

108. Liggett SB, Cresci S, Kelly RJ, et al. A GRK5 polymorphism that inhibits beta-adrenergic receptor signaling is protective in heart failure. *Nat Med*. 2008;14:510-517.

109. A.N. Aggarwal, K. Chaudhry, S.K. Chhabra, G.A. D'Souza, D. Gupta, S.K. Jindal. Prevalence and Risk Factors for Bronchial Asthma in Indian Adults: A Multicentre Study. Indian J Chest Dis Allied Sci 2006; 48: 13-22

110. J. Sunyer, J.M. Antó, M. Kogevinas et al. Risk factors for asthma in young adults . Eur Respir J 1997; 10: 2490–2494

111. Dursun AB ,Kurt OK, Bayiz H, Ozkan E, Cakaloglu A, Karasoy D. Does gender affect asthma control in adult asthmatics?. Chron Respir Dis. 2014 ;11(2):83-7

112. Mosen DM, Schatz M, Magid DJ et al.The relationship between obesity and asthma severity and control in adults. J Allergy Clin Immunol. 2008; 122(3):507-11.

113. Shore SA. Obesity and asthma: possible mechanisms. J Allergy Clin Immunol. 2008;121(5):1087-93

114. Ismaila A, Corriveau D, Vaillancourt J. Impact of adherence to treatment with fluticasone propionate/salmeterol in asthma patients. Curr Med Res Opin. 2014 Jul;30(7):1417-25.

115. K.R. Chapman, L.P. Boulet, R.M. Rea , E. Franssen . Suboptimal asthma control: prevalence, detection and consequences in general practice . Eur Respir J 2008; 31: 320–325

116. N. C. Thomson, R. Chaudhuri and E. Livingston. Active cigarette smoking and asthma .Clin Exp Allergy 2003; 33:1471–1475

117. Simpson WG. Gastroesophageal reflux disease and asthma: diagnosis and management. Arch Intern Med. 1995; 155:798-804.

118. Nelson HS, weiss St, Bleecker er, Yancey SW, Dorinsky PM. The Salmeterol multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. Chest 2006;129:15-26

119. Walker et al. G protein coupled receptor kinase 5 regulates airway response induced by muscarinic receptor activation. A J Physiol Lung Cell Mol Physiol. 2004 ; 286:312-9.

Image.1: Genomic DNA Agarose gel electrophoresis

Image shows 0.8% agarose gel electrophoresis of the genomic DNA. Lanes shows the extracted DNA under UV transillumination on ethidium bromide Staining

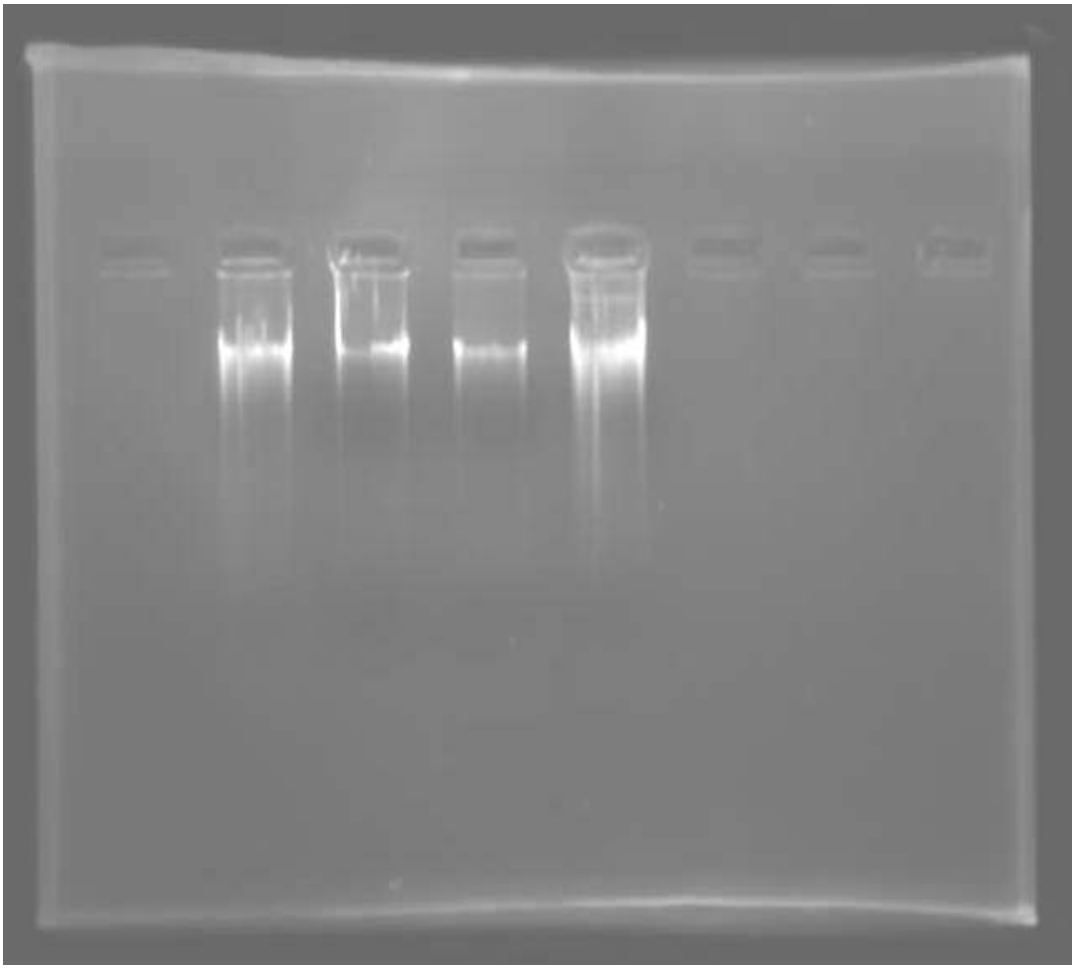


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Image.2: 2% Agarose gel electrophoresis of PCR Product

Image shows 2% agarose gel electrophoresis of the PCR amplified DNA. Lane 1 shows the DNA ladder. Lanes 2-7 shows the PCR DNA product of size 381 bp

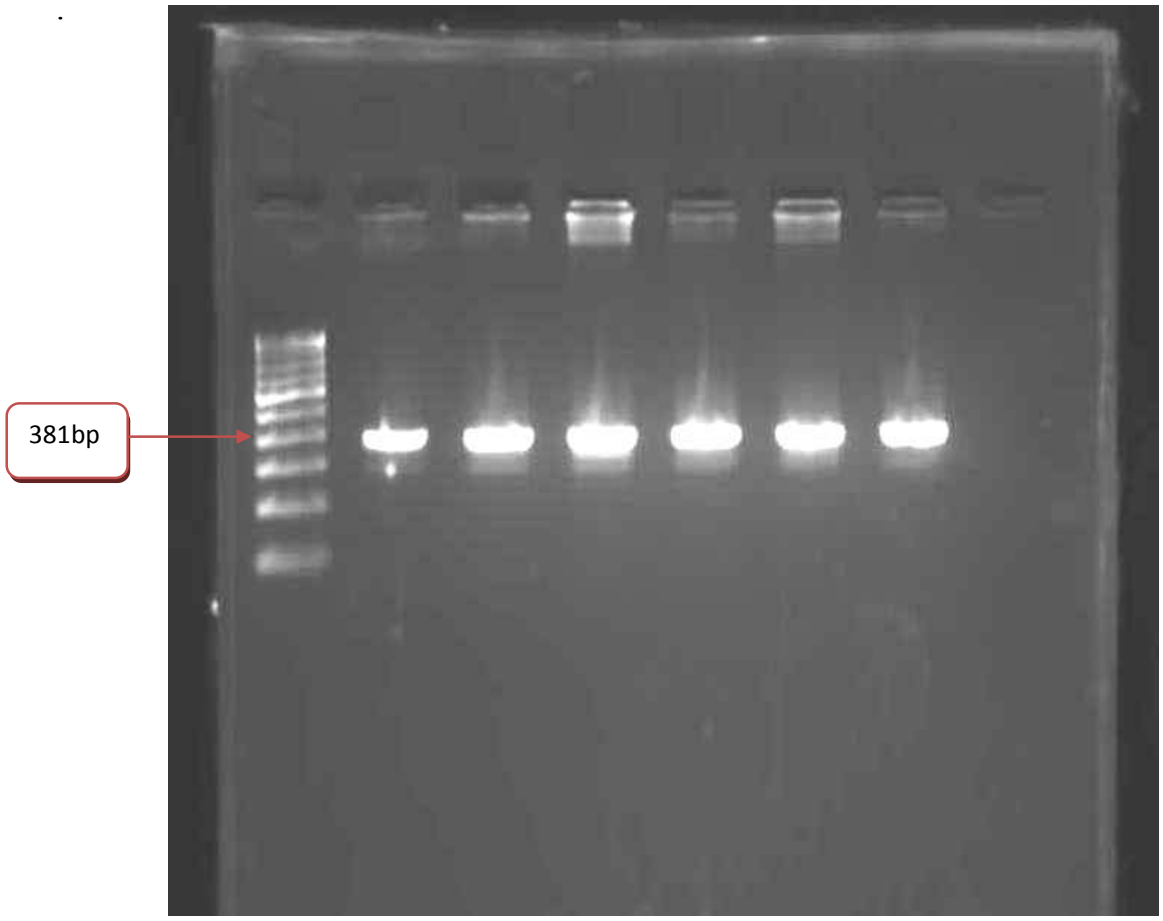
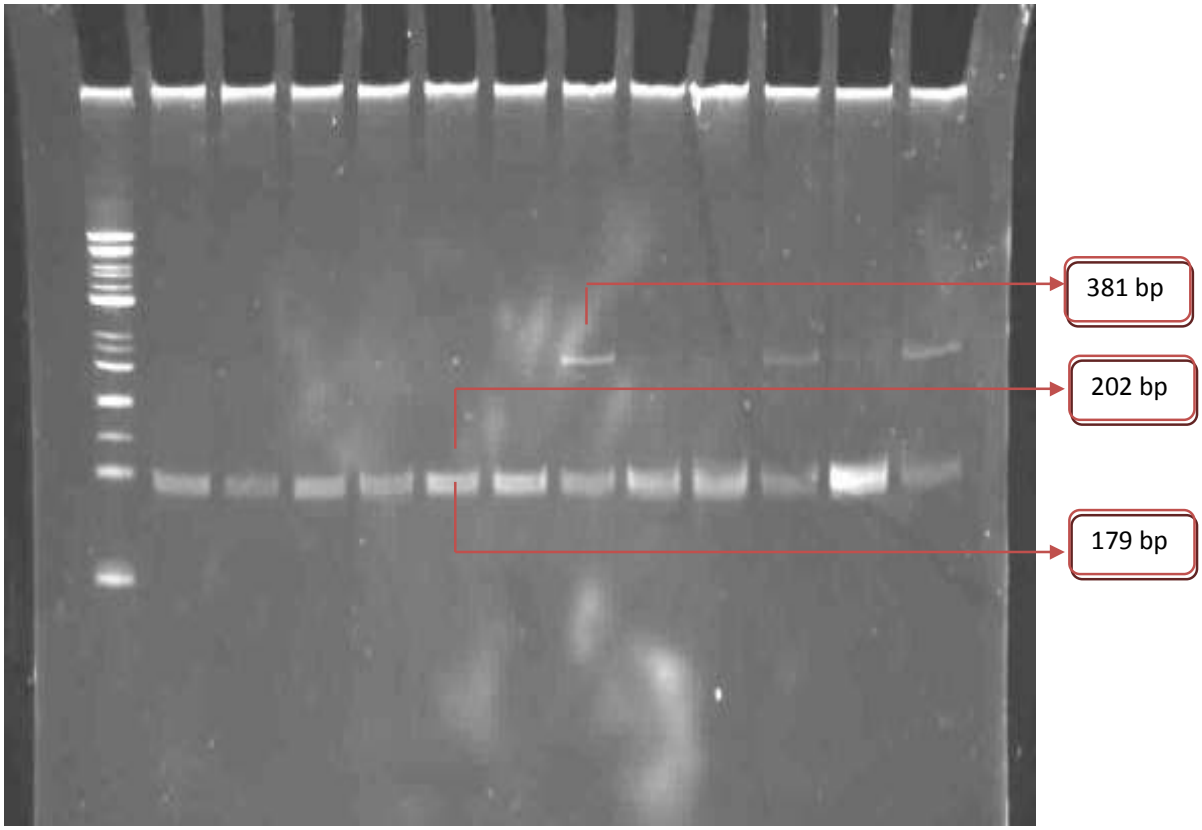


Image.3: 15% Polyacrylamide gel electrophoresis of restriction digestion product

Lane1 shows the DNA ladder. Lanes 8, 11, 13 shows a heterozygous product. All other lane shows wild type homozygous product.



ABBREVATIONS

WHO	-	World Health Organization
COPD	-	chronic obstructive pulmonary disease
SNP	-	*Single Nucleotide Polymorphism*
GRK	-	G protein-coupled receptor kinase
GPCR	-	*G protein-coupled receptors*
BTS	-	*British Thoracic Society*
NHLBI	-	*NIH Heart, Lung and Blood Institute*
GINA	-	Global Initiative for Asthma
IgE	-	Immunoglubulin E
PG	-	prostaglandin
LOX	-	Lipoxygenase
ASM	-	Airway smooth muscle
TH $_{1\ \&\ 2}$	–	T-Helper cells $_{1\&2}$
AHR	–	Airway Hyperresponsiveness
NF-KB	-	nuclear factor – kappa beta
GATA-3	-	Trans-acting T-cell-specific transcription factor
PEFR	-	Peak Expiratory Flow Rate
BA	-	Bronchial Asthma

PSG Institute of Medical Science and Research, Coimbatore
Institutional Human Ethics Committee
INFORMED CONSENT FOR RESEARCH PROJECTS

I am **Dr.R.Keerthana Brattiya** carrying out a study on the topic**: Association between GRK5 polymorphism and poorly controlled asthma**

As part of my research project being carried out under the aegis of the Department of: **Pharmacology**

My research guide is**: Prof.S.Ramalingam**

The justification for this study is: to identify whether genetic polymorphism has effect on the control of asthma as a result in future my study will help them identify the appropriate group of drugs for their effective control

The objectives of this study are:
To study the risk factors associated with poorly controlled asthma
To study genetic association (GRK5 Leu 41 polymorphism) and control (i.e. the therapeutic response) in bronchial asthma.

Sample size: 150 patients

Study participants are: Asthma patients above the age of 18

Location: PSGIMSR, Coimbatore

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

Initial interview :10 to 15 minutes.

Data collected will be stored for a period of fifteen years. We will / will not use the data as part of another study.

Blood sample collection: 2 ml.

No. of times it will be collected: **Once**

Whether blood sample collection is part of routine procedure or for research (study) purpose: **Research** purpose

Specify **purpose**, discomfort likely to be felt and side effects, if any: To do genetic analysis and no discomfort or side effects

Whether blood sample collected will be stored after study period**: No,** it will be destroyed

Whether blood sample collected will be sold: **No**

Whether blood sample collected will be shared with persons from another institution: **No**

Medication given, if any, duration, side effects, purpose, benefits**: No medications**

Benefits from this study: To identify whether the patient has the polymorphism and that is the cause for his poor control of asthma

Risks involved by participating in this study : **No risks**

How the **results** will be used: the results will be used for **further researches and publications**

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services

offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date:

Witness:

Association between GRK5 Polymorphism and poorly controlled asthma

Dr.R.Keerthana Brattiya Dr.Ramalingam

PSGIMSR

Case Report Form

Patient name: IP/OP no:

Age/Gender: Address:

Height: Weight: BMI:

Occupation – (Current &Past):

Any Hazardous exposure in work place:

Ethnicity (Religion/caste):

H/o known allergens:

Personal History:

Smoking –(cigar/beedi, How many, How long)

Alcohol-

Other Sub Abuse-

H/o exposure to irritants (paint fumes , household sprays)

Family History

(H/O, asthma, atopic dermatitis,Eczema, allergic rhinitis)

Disease related details

1)Asthma-> Duration of disease

Treatment

2) Co morbid conditions & treatment (Drugs like beta blockers, Aspirin)

HT

DM

TB

Others

3) No of emergency visits in last year

4) No of hospitalization in last year

5) During past 4 weeks have you had

-Respiratory Infection

-GERD symptoms

-Rhino sinusitis

-Pets

Patient's Name:_____ Today's Date:_____

Asthma Control Test™ (ACT) is:

- A quick test that provides a numerical score to assess asthma control.
- Recognized by the National Institutes of Health (NIH) in its 2007 asthma guidelines.[1]
- Clinically validated against spirometry and specialist assessment.[2]

PATIENTS:

1. Answer each question and write the answer number in the box to the right of each question.
2. Add your answers and write your total score in the TOTAL box shown below.
3. Discuss your results with your doctor.

						SCORE
1. In the past **4 weeks**, how much of the time did your **asthma** keep you from getting as much done at work, school or at home?	All of the time 1	Most of the time 2	Some of the time 3	A little of the time 4	None of the time 5	
2. During the past **4 weeks**, how often have you had shortness of breath?	More than once a day 1	Once a day 2	3 to 6 times a week 3	Once or twice a week 4	Not at all 5	
3. During the past **4 weeks**, how often did your **asthma** symptoms (wheezing, coughing, shortness of breath, chest tightness or pain) wake you up at night or earlier than usual in the morning?	4 or more nights a week 1	2 or 3 nights a week 2	Once a week 3	Once or twice 4	Not at all 5	
4. During the past **4 weeks**, how often have you used your rescue inhaler or nebulizer medication (such as albuterol)?	3 or more times per day 1	1 or 2 times per day 2	2 or 3 times per week 3	Once a week or less 4	Not at all 5	
5. How would you rate your **asthma** control during the **past 4 weeks**?	Not controlled at all 1	Poorly controlled 2	Somewhat controlled 3	Well controlled 4	Completely controlled 5	
						TOTAL

If your score is 19 or less, your asthma may not be under control.

Copyright 2002, by QualityMetric Incorporated.
Asthma Control Test is a trademark of QualityMetric Incorporated.
The Asthma Control Test is for people with asthma 12 years and older.

HEALTHCARE PROVIDER:

- Include the ACT score in your patient's chart to track asthma control.

References: 1. US Department of Health and Human Services, National Institutes of Health, National Heart, Lung and Blood Institute. *Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma (EPR-3 2007).* NIH Item No. 08-4051. http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.htm. Accessed September 10, 2007. **2.** Nathan RA et al. *J Allergy Clin Immunol.* 2004;113:59-65.

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ஆஸ்துமா கட்டுப்பாடு பரிசோதனை

1.கடந்த 4 வாரங்களில், ஆஸ்துமாவினால் தாங்கள், அலுவலகம், பள்ளி அல்லது வீட்டில் எவ்வளவு நேரம் வேலை செய்ய இயலாமல் போனது ?

1 அனைத்து நேரமும்

.2. பெரும்பாலான நேரம்

3. சில நேரம்

4. மிக சிறிய நேரம்

5. முற்றிலும் இல்லை

2. கடந்த 4 வாரங்களின் போது, எத்தனை முறை மூச்சுத்திணறல் இருந்தது?

1. ஒரு நாளில் ஒரு முறைக்கு மேல்

2. ஒரு நாளில் ஒரு முறை

3. ஒரு வாரத்தில் 3 முதல் 6 முறை

4. ஒரு வாரத்தில் 1 அல்லது 2 முறை

5. முற்றிலும் இல்லை

3. கடந்த 4 வாரங்களின் போது, எத்தனை முறை ஆஸ்த்மாவின் அறிகுறிகள் ஆன முச்சிதிணறல் இருமல், நெஞ்சு இறுக்கம் அல்லது வலி , இவற்றின் காரணமாக இரவு அல்லது விடியற் காலையில் வழக்கத்திற்கு மாறாக நீங்கள் படுக்கையில் இருந்து எழுந்து கொள்ள நேரிட்டது ?

1. ஒரு வாரத்தில் 4 அல்லது அதற்கு மேற்பட்ட இரவுகளில்

2. ஒரு வாரத்தில் 2 அல்லது 3 இரவுகள்

3. வாரம் ஒரு முறை

4. ஒருமுறை அல்லது இருமுறை 4 வாரங்களில்

5. முற்றிலும் இல்லை

4. கடந்த 4 வாரங்களில் போது, நீங்கள் உங்கள் மீட்பு இன்ஹேலர் அல்லது nebulizer மருந்து (அதாவது செரோப்லோ அஸ்தலின் போன்ற) எத்தனை முறை பயன்படுத்தினீர்கள்?

1. ஒரு நாளைக்கு 3 அல்லது அதற்கு மேற்பட்ட முறை

2. நாளொன்றுக்கு 1 அல்லது 2 முறை

3. வாரத்திற்கு 2 அல்லது 3 முறை

4. வாரத்திற்கு 1 முறை

5. முற்றிலும் இல்லை

5. கடந்த 4 வாரங்களின் போது
உங்கள் ஆஸ்த்மாவின் கட்டுபாட்டை நீங்கள் எப்படி மதிப்பிடுவீர்கள் ?

1. முற்றிலும் கட்டுப்பாட்டில் இல்லை

2. மோசமான கட்டுப்பாடு

3. ஓரளவு கட்டுப்பாடு

4. நன்கு கட்டுப்பாடு

5. முழுமையான கட்டுப்பாடு **Total score :**

xg;Gjy; gbtk;

,uh. fPh;j;jdh gpuhl;bah Mfpa ehd; PSG kUj;Jtf; fy;Yhupapd; Pharmacology Jiwapd; fPo;> GRK 5 ghypkhh;gp]k; kw;Wk; Nkhrkhf fl;Lg;gLj;jg;gl;l M];Jkh ,ilNa cs;s njhlh;G vd;w jiyg;gpy; Ma;T Nkw;nfhs;s cs;Nsd;.

vd; Ma;T topfhl;b: Nguhrpupah;. R. ,uhkypq;fk;

Ma;T Nkw;nfhs;tjw;fhd mbg;gil: GRK 5 vd;w kugZtpdhy; M];Jkhtpd; fl;Lg;ghL Fiwfpwjh vd;gij mwpa

Ma;tpd; Nehf;fk;: kugZtpd; khw;wj;jpw;Fk; M];Jkhtpd; fl;Lg;ghl;bw;Fk; cs;s njhlh;G

Ma;T Nkw;nfhs;Sk; ,lk;: PSG kUj;Jt fy;Yhhp kUj;Jtkid

Ma;T gyd;fs;: tUk; fhyq;fspd; vd; Ma;tpdhy; rpwe;j Kiwapy; M];Jkhit fl;Lg;gLj;j ,aYk;

,e;j Ma;tpy; fpilf;Fk; jfty;fs; 5 tUlq;fs; ghJfhf;fg;gLk;. ,it NtW ve;j Ma;tpw;Fk; gad;gLj;jg; gl khl;lhJ. ve;j epiyapYk; cq;fisg; gw;wpa jfty;fs; ahUf;Fk; njhptpf;fg;gl khl;lhJ. mit ,ufrpakhf itf;fg;gLk;.

,e;j Ma;tpy; gq;Nfw;f xg;Gf;nfhs;Stjhy; ve;j tpjkhd gyDk; cq;fSf;F fpilf;fhJ. ve;j Neuj;jpy; Ntz;LkhdhYk; Ma;tpypUe;J tpyfpf;nfhs;Sk; cupik cq;fSf;F cz;L. Ma;tpypUe;J tpyfpf;nfhs;tjhy; cq;fSf;F mspf;fg;gLk; rpfpr;irapy; ve;j tpj khw;wKk; ,Uf;fhJ.

,e;j Muha;r;rpf;fhf cq;fsplk; rpy Nfs;tpfs; Nfl;fg;gLk;. 2ml ,uj;j khjpupfs; vLf;fg;gLk;.

NkYk;> ,e;j Ma;tpy; gq;F nfhs;tJ cq;fs; nrhe;j tpUg;gk;. ,jpy; ve;j tpjf; fl;lhaKk; ,y;iy. ePq;fs; tpUg;gg; gl;lhy;> ,e;j Ma;tpd; KbTfs; cq;fSf;Fj; njhpag;gLj;jg;gLk;.

Ma;thshpd; ifnahg;gk; /Njjp :

Ma;Tf;Fl;gLgthpd; xg;Gjy; :

ehd; ,e;j Muha;r;rpapd; Nehf;fk; kw;Wk; mjd; gad;ghl;bidg; gw;wp njspthfTk;> tpsf;fkhfTk; njhpag;gLj;jg; gl;Ls;Nsd;. ,e;j Muha;r;rpapy; gq;F nfhs;sTk;. ,e;j Muha;r;rpapd; kUj;Jt uPjpahd Fwpg;Gfis tUk; fhyj;jpYk; cgNahfg;gLj;jpf; nfhs;sTk; KO kdJld; rk;kjpf;fpNwd;.

Ma;Tf;Fl;gLgthpd; ngah;> Kfthp :

ifnahg;gk; & Njjp

MASTER CHART

ID NO	AGE	SEX	CONTROL	SNP
BAS 1	21	F	P	WILD HOMO
BAS 2	44	F	P	WILD HOMO
BAS 3	37	F	W	WILD HOMO
BAS 4	21	M	W	WILD HOMO
BAS 5	67	F	W	WILD HOMO
BAS 6	56	F	W	WILD HOMO
BAS 7	52	M	W	HETERO
BAS 8	32	F	P	WILD HOMO
BAS 9	65	F	W	WILD HOMO
BAS 10	31	F	W	HETERO
BAS 11	21	M	P	WILD HOMO
BAS 12	39	M	P	WILD HOMO
BAS 13	42	F	P	WILD HOMO
BAS 14	51	F	W	WILD HOMO
BAS 15	34	M	P	WILD HOMO
BAS 16	69	M	W	HETERO
BAS 17	54	F	P	WILD HOMO
BAS 18	69	F	W	WILD HOMO
BAS 19	33	F	W	WILD HOMO
BAS 20	77	F	P	WILD HOMO
BAS 21	47	M	W	WILD HOMO
BAS 22	60	F	W	WILD HOMO
BAS 23	52	F	P	WILD HOMO
BAS 24	35	M	W	WILD HOMO
BAS 25	54	F	P	WILD HOMO
BAS 26	70	F	P	WILD HOMO
BAS 27	76	F	P	WILD HOMO
BAS 28	58	M	P	WILD HOMO
BAS 29	71	M	W	WILD HOMO
BAS 30	40	F	W	WILD HOMO
BAS 31	47	M	W	WILD HOMO
BAS 32	65	F	W	WILD HOMO
BAS 33	40	F	W	WILD HOMO
BAS 34	24	M	P	WILD HOMO
BAS 35	23	F	P	WILD HOMO
BAS 36	49	M	P	WILD HOMO
BAS 37	61	F	W	WILD HOMO
BAS 38	57	F	P	WILD HOMO
BAS 39	64	M	W	WILD HOMO
BAS 40	20	F	W	WILD HOMO
BAS 41	35	F	W	HETERO

MASTER CHART

BAS 42	32	M	W	WILD HOMO
BAS 43	24	F	W	WILD HOMO
BAS 44	43	F	W	WILD HOMO
BAS 45	57	M	W	WILD HOMO
BAS 46	36	F	P	WILD HOMO
BAS 47	55	F	P	WILD HOMO
BAS 48	45	F	W	WILD HOMO
BAS 49	80	F	P	WILD HOMO
BAS 50	23	F	W	WILD HOMO
BAS 51	32	F	W	HETERO
BAS 52	40	M	W	WILD HOMO
BAS 53	55	F	P	WILD HOMO
BAS 54	32	F	W	WILD HOMO
BAS 55	35	F	W	HETERO
BAS 56	63	M	P	WILD HOMO
BAS 57	49	F	W	WILD HOMO
BAS 58	31	F	W	WILD HOMO
BAS 59	42	M	W	HETERO
BAS 60	72	M	W	WILD HOMO
BAS 61	55	F	W	WILD HOMO
BAS 62	30	F	P	WILD HOMO
BAS 63	50	F	W	HETERO
BAS 64	52	F	W	WILD HOMO
BAS 65	60	F	W	WILD HOMO
BAS 66	53	F	W	WILD HOMO
BAS 67	38	F	W	WILD HOMO
BAS 68	41	F	W	WILD HOMO
BAS 69	32	F	W	WILD HOMO
BAS 70	27	M	W	WILD HOMO
BAS 71	63	F	P	WILD HOMO
BAS 72	72	M	W	WILD HOMO
BAS 73	47	F	W	HETERO
BAS 74	43	F	P	WILD HOMO
BAS 75	35	F	W	HETERO
BAS 76	60	F	P	WILD HOMO
BAS 77	55	F	W	WILD HOMO
BAS 78	65	M	W	WILD HOMO
BAS 79	52	F	W	WILD HOMO
BAS 80	36	F	W	WILD HOMO
BAS 81	43	M	W	WILD HOMO
BAS 82	39	M	W	WILD HOMO
BAS 83	26	M	P	WILD HOMO

MASTER CHART

BAS 84	48	F	W	HETERO
BAS 85	26	F	W	WILD HOMO
BAS 86	42	F	W	WILD HOMO
BAS 87	26	F	W	WILD HOMO
BAS 88	28	F	W	WILD HOMO
BAS 89	49	F	W	WILD HOMO
BAS 90	60	F	P	WILD HOMO
BAS 91	60	F	W	WILD HOMO
BAS 92	29	F	P	WILD HOMO
BAS 93	57	F	W	WILD HOMO
BAS 94	67	M	P	WILD HOMO
BAS 95	25	M	W	WILD HOMO
BAS 96	59	F	W	WILD HOMO
BAS 97	39	M	P	WILD HOMO
BAS 98	48	F	W	WILD HOMO
BAS 99	21	M	W	WILD HOMO
BAS 100	47	M	P	WILD HOMO
BAS 101	65	F	W	WILD HOMO
BAS 102	24	F	W	WILD HOMO
BAS 103	28	F	W	WILD HOMO
BAS 104	69	F	W	WILD HOMO
BAS 105	52	M	P	WILD HOMO
BAS 106	42	M	P	WILD MUTANT
BAS 107	83	M	P	HETERO
BAS 108	63	M	W	WILD HOMO
BAS 109	52	F	P	WILD HOMO
BAS 110	36	F	W	WILD HOMO
BAS 111	64	M	W	HETERO
BAS 112	65	F	P	WILD HOMO
BAS 113	64	M	W	WILD HOMO
BAS 114	42	F	W	WILD HOMO
BAS 115	80	F	W	WILD HOMO
BAS 116	65	F	W	WILD HOMO
BAS 117	46	F	W	WILD HOMO
BAS 118	26	F	P	WILD HOMO
BAS 119	50	M	P	WILD HOMO
BAS 120	50	M	W	WILD HOMO
BAS 121	34	M	P	WILD HOMO
BAS 122	50	F	P	WILD HOMO
BAS 123	49	F	P	WILD HOMO
BAS 124	74	F	P	WILD HOMO

MASTER CHART

BAS 125	47	M	P	WILD HOMO
BAS 126	32	M	P	HETERO
BAS 127	62	F	P	WILD HOMO
BAS 128	42	F	P	HETERO
BAS 129	63	F	P	WILD HOMO
BAS 130	52	F	P	HETERO
BAS 131	55	F	P	WILD HOMO
BAS 132	65	M	P	HETERO
BAS 133	59	M	P	WILD HOMO
BAS 134	37	F	P	WILD HOMO
BAS 135	45	M	P	WILD HOMO
BAS 136	58	F	P	HETERO
BAS 137	19	M	P	WILD HOMO
BAS 138	32	M	P	WILD HOMO
BAS 139	71	M	P	WILD HOMO
BAS 140	69	F	P	HETERO
BAS 141	22	F	P	HETERO
BAS 142	45	F	P	WILD HOMO
BAS 143	59	F	P	WILD HOMO
BAS 144	71	F	P	WILD HOMO
BAS 145	35	F	P	WILD HOMO
BAS 146	50	F	P	WILD HOMO
BAS 147	65	F	P	WILD HOMO
BAS 148	22	F	P	WILD HOMO
BAS 149	63	F	P	HETERO
BAS 150	21	M	P	WILD HOMO
BAS 151	53	F	P	WILD HOMO
BAS 152	43	F	P	WILD HOMO
BAS 153	26	F	P	WILD HOMO
BAS 154	29	M	P	WILD HOMO
BAS 155	69	F	P	HETERO
BAS 156	31	F	P	WILD HOMO
BAS 157	77	M	P	WILD HOMO
BAS 158	33	F	P	WILD HOMO
BAS 159	46	F	P	WILD HOMO
BAS 160	63	F	P	WILD HOMO